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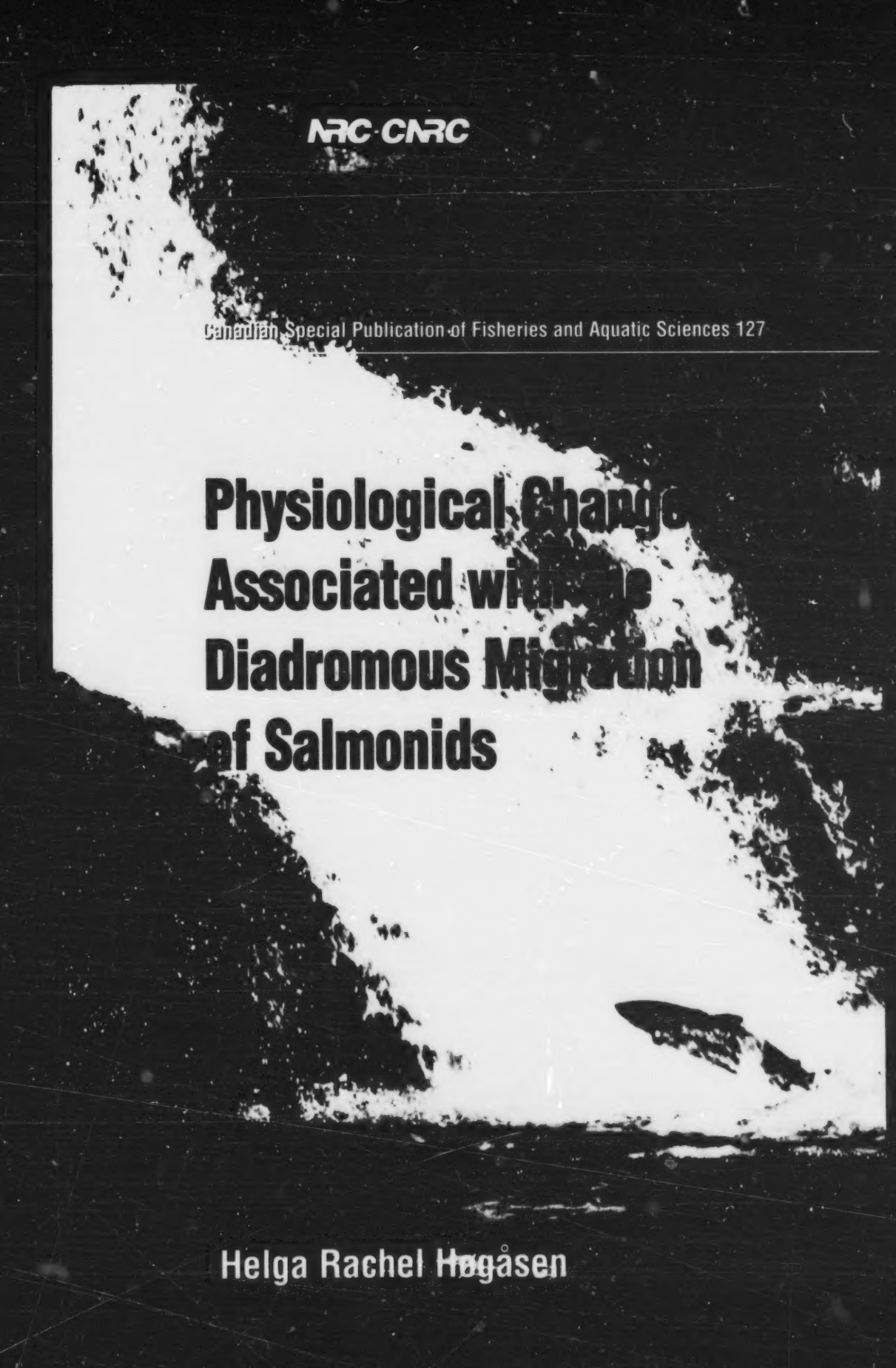
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Physiological Changes Associated with the Diadromous Migration of Salmonids
Helga Rachel Høgåsen

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Physiological Changes Associated with the Diadromous Migration of Salmonids

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Physiological Changes Associated with the Diadromous Migration of Salmonids

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Abstract

The book reviews and discusses present knowledge concerning the diadromous migration of salmonids. It groups elements ranging from ecology to cell biology, to give the reader a background knowledge for critical understanding of published literature and for proper design of experiments. In the first chapter, elements related to the river migration are discussed. These include abiotic and biological factors involved in onset of migration, swimming activity during migration, metabolic aspects, and possible mechanisms for orientation. In the second chapter, structural and physiological changes associated with the transfer between different salinities are described. These include adjustments in water and ion balance, as well as cardiovascular, respiratory, and metabolic changes. In the third chapter, elements of preadaptation to these transfers are reviewed. Comparative aspects between different salmonid species are exposed. The interrelation between smoltification and migration is discussed. The existence of changes in hormone production, metabolism, distribution, and effect during smoltification is underlined. The presence of a preadaptation to freshwater transfer and its putative relation to desmoltification are discussed. An evolutionary hypothesis by which new pathways for inhibition of desmoltification allowed some salmonids to remain in the sea longer is proposed. In the fourth and main chapter of the book, endocrinological aspects are reviewed, with emphasis on thyroid hormones, corticosteroids, and prolactin. For each hormone (group), general knowledge on its synthesis, regulation, metabolism, distribution, and action in fish is reviewed, and its putative involvement in migration, preadaptory, and adaptory changes related to salinity transfer is discussed. The diversity and plasticity of salmonids are underlined.

Résumé

L'auteur passe en revue et traite des connaissances actuelles sur la migration diadrome des salmonidés. Des éléments de disciplines complémentaires, allant de l'écologie à la biologie cellulaire, sont regroupés afin de donner au lecteur les connaissances fondamentales lui permettant de comprendre les informations essentielles des communications scientifiques et de concevoir des expériences de façon appropriée. Le premier chapitre traite des éléments connexes à la migration en cours d'eau, notamment des facteurs abiotiques et biotiques amorçant les migrations, les activités de nage pendant les migrations, les aspects métaboliques et les mécanismes d'orientation possibles. Le deuxième traite des modifications structurales et physiologiques liées au déplacement entre des milieux de salinité différente, notamment les adaptations des équilibres hydriques et ioniques et les modifications cardio-vasculaires, respiratoires et métaboliques. Le troisième chapitre passe en revue les éléments de préadaptation à ces déplacements. On y présente des comparaisons entre les diverses espèces de salmonidés, traite des relations entre la smoltification et la migration et souligne l'existence de changements affectant la production hormonale, le métabolisme, la répartition et les effets de différentes hormones pendant la smoltification. L'existence d'une préadaptation au déplacement vers l'eau douce et sa relation supposée avec la désmoltification font l'objet d'une discussion. L'auteur propose une hypothèse évolutive faisant appel à de nouvelles voies d'inhibition de la désmoltification permettant à certains salmonidés de demeurer en mer plus longtemps. Le quatrième et principal chapitre comporte un examen des aspects endocriniens. L'accent est mis sur les hormones thyroïdiennes, les corticostéroïdes et la prolactine. On trouve, pour chaque hormone (ou groupe) des connaissances générales sur sa synthèse, sa régulation, son métabolisme, sa répartition et son effet chez les poissons ainsi qu'une discussion de son effet supposé sur la migration et de ses effets préadaptatifs et adaptatifs dans le contexte du déplacement vers un milieu de salinité différente. La diversité et la plasticité des salmonidés sont soulignées.

Introduction

Salmonids undertake several types of migrations during their lifetime. Their eggs are usually buried in gravel and the newly hatched young remain in this gravel until they have absorbed most of their yolk. The young fish then emerge from the gravel and swim up to the water surface to gulp a mouthful of air, filling their swim bladder so that they may acquire an appropriate buoyancy (Saunders 1965). Although short, this procedure may be considered as a true migration (Smith 1985) because (*a*) it is a precisely timed and orientated movement from one habitat, the gravel, to another, open water, and (*b*) it is related to a definite physiological requirement, filling their swim bladder with air. After this first migration, the young move to a nursery habitat, usually more quiet and productive than the spawning area. This gravel-to-nursery migration may vary from a few metres between the gravel and a quiet part of the natal stream to a migration of many kilometres to lower parts of the river (Murphy et al. 1997) or to the sea. Following a period of a few months to several years in the nursery area(s), freshwater-living juveniles move to seawater feeding habitats, which may be more than 6000 km away from their home river. Then, after a few weeks to several years, salmonids return to their home river to spawn and (or) to overwinter. After spawning, some salmon may undertake a last short-scale migration away from the spawning ground before dying (Baglinière et al. 1990). Others may continue migrating between freshwater and sea, sometimes several times.

This book deals with the migration that leads the salmonids from a freshwater habitat to the sea and the return migration to freshwater. Despite the great ecological and economical interests of this migration, its physiological regulation is still poorly understood. Studying the physiology of migration of salmonids is indeed a complex area of research for several reasons. First, it covers a wide range of different fields from ecology to cellular biology. Second, changes associated with migration in salmonids are often intermingled with other major physiological processes, such as the parr–smolt transformation, sexual maturation, and the adaptation to a different water salinity. These processes change the physiology of the studied fish during the course of its migration; thus, several dynamic processes must be considered together. Third, migratory behavior of salmonids is amenable to local adaptations, which reflect the complexity of its regulatory pathway. Some populations may show a high plasticity in migratory behavior, and a number of different life-strategies have evolved in different species and stocks. In particular, the time relation between migration and parr–smolt transformation or sexual maturation differs among stocks. Finally, fish are easily stressed by human manipulation. Chronic cannulation of fish may represent a valuable solution for reducing stress in a number of physiological studies but is rather poorly fitted to studying migrating animals in the wild. Laboratory approaches, allowing for controlled conditions and minimally stressing sampling protocols, may provide valuable information on the physiology of migrating salmonids. However, if the results are to be applicable in the wild, both design of experiments and interpretation of results should take into account the many variables known to affect wild salmonids during the migration period.

In this book, an attempt has been made to group elements ranging from ecology to cell biology, which may be of importance when designing an experiment or interpreting data on migration. The intention was to provide easily accessible data and references in the many fields necessary for studying the physiology of migration and in this way to facilitate integrative approaches. In the first part, elements concerning the onset of migration, the motor activity during migration, metabolic effects of migration, and the cues used for orientation are reviewed. In the second part, changes associated with the transfer between freshwater

and seawater are exposed. In the third part, the elements of preadaptation both to seawater transfer and freshwater transfer are discussed. In the fourth and main part of this book, elements of endocrinology related to migration of salmonids are reviewed. Special attention has been given to three hormones or group of hormones that seem to play a central role during migration, the thyroid hormones, corticosteroids, and prolactin. To avoid repetition, endocrinological aspects are mentioned only briefly in the first three parts of the book.

1. The river migration

This chapter deals with the freshwater part of the two main migrations undertaken by salmonids, leading them to the sea or back to freshwater. A number of external and internal factors that are thought to regulate onset of migration are first discussed. Then, knowledge concerning the motor activity of fish during migration and the physiological adjustments associated with bursts of swimming are exposed. An estimation of metabolic needs and the ways by which these are covered are discussed. Finally, putative mechanisms used for orientation during river migration are presented.

1.1. Factors regulating onset of migration

Onset of migration is associated with striking contrasts. Among different species, the time spent in freshwater before salmonids undertake their first migration to the sea may vary from a few weeks to many years (Randall et al. 1987). On the other hand, all smolts of sockeye salmon, *O. nerka*, in a lake may start to migrate on the same day (Smith 1985). The aim of the present chapter was to try to understand how such contrasting events are regulated.

A number of factors are thought to be involved in regulating onset of migration. These factors may act at two levels. First, they may regulate the time necessary for the fish to reach a physiological state of "migratory readiness" (Baggerman 1960). Second, they may act as triggering factors to induce migration in those fish that are ready to migrate. These have been grouped into abiotic and biotic factors. Their relative importance seems to be species, place, and time dependent. Numerous negative results concerning their effect on migration exist in the literature. These are not exposed in the present review, which is aimed at listing some of the factors that may, under certain circumstances, influence migration. It is therefore important not to generalize from the reported examples but rather remember that significant variations may exist between species, stocks, individuals, and years.

Available knowledge concerning development of migratory readiness and onset of migration in salmonids mainly concerns seaward migration of smolts. Regulation of the return migration of salmonids has received less attention. Difficulties associated with studies in the open sea are probably one reason for this. The close association between sexual maturation and upstream migration in Atlantic salmon, *Salmo salar*, and Pacific salmon species, *Oncorhynchus* spp., suggesting an obvious clue to the motivation to migrate, may be another reason for the few studies dealing with the determinants of upstream migration in adult salmonids.

1.1.1. Abiotic factors

1.1.1.1. Photoperiod

Among salmonids, photoperiod is considered the most usual synchronizer of seasonally changing physiological processes such as sexual maturation, spawning, growth, and migration (Hoar 1988). Increasing daylength artificially several months before the normal schedule may induce earlier downstream migration (Zaugg and Wagner 1973; Wagner 1974). Removing the normal increase in photoperiod by exposure to constant photoperiod may in turn delay downstream migratory behavior (Wagner 1974; Isaksson 1976).

In addition to following a seasonal rhythm, migratory behavior follows a daily rhythm, downstream migration occurring predominantly at night (Thorpe et al. 1988; Northcote 1984). This pattern may, however, be less distinct in highly turbid rivers (Northcote 1984)

or at the end of the migrating season (Fängstam et al. 1993; Moore et al. 1995), and the pattern of hourly migration within a single population may differ significantly among years (Rottiers and Redell 1993). Greenstreet (1992*a*) showed that downstream migration of Atlantic salmon, *Salmo salar*, increased when light intensity was below a threshold level of 10 lux but suggested that geographical variations in the light intensity response threshold could exist.

The photoperiodic cycle provides a unique cue for seasonally rhythmic biological activities because of its predictable nature, in contrast to the more variable temperature cycles. Night migration may offer some advantage by maximizing predator avoidance (Fängstam et al. 1993; Greenstreet 1992*a*). This might, however, be less important in turbid rivers, or at the end of the migration season, when the establishment of larger schools (Fängstam et al. 1993) may offer sufficient protection against predation.

Putative physiological mechanisms by which photoperiod influences migration include endogenous effects and immediate consequences of changes in light condition. Photoperiodic changes in light intensity induce changes in melatonin production, which may in turn influence the swimming behavior and excitability of the fish (Zachmann et al. 1992). Melatonin production in fish also depends on water temperature (Zachmann et al. 1992) and different combinations of photoperiod and temperature may cause different rhythmic patterns of migration at different periods or in consecutive years. Atlantic salmon have been shown to become increasingly diurnal in their activity as the temperature rises (Fraser et al. 1993) and such a mechanism could explain increased diurnal migration at the end of the migration season. Photoperiod may also indirectly regulate onset of downstream migration by affecting smoltification (Hoar 1988). Several studies have implicated photoperiod in controlling the timing of smoltification and continuous light is able to inhibit development of salinity tolerance (McCormick et al. 1987). Similarly, photoperiod may indirectly regulate upstream migration by affecting gonadotropin secretion (Hasler and Scholz 1983). Finally, night migration of smolts may result from their inability to maintain position in the dark (Thorpe 1988). This is consistent with the observations that downstream migration during the day increases in turbid waters, while downstream migration during the night decreases when the river is illuminated either artificially or by the moon (Hansen and Jonsson 1985; Thorpe et al. 1988). Darkness severely decreases the critical swimming speed of fish, particularly small ones (Hammer 1995). It has been proposed that smolts may be particularly unable to maintain a visual fix in the dark as a result of changes in retinal pigments (Bridges and Delisle 1974; Hasler and Scholz 1983). However, the increased diurnal migratory activity at the end of the migration season can not be explained by this direct effect of illumination. This suggests that several physiological mechanisms are involved.

1.1.1.2. Temperature

The seasonal occurrence and geographic distribution of anadromy among salmonids suggests that migration occurs only within a specific range of temperatures. Both too high and too low temperatures seem to inhibit migratory behavior.

Within each salmonid species, there seems to be an increasing degree of anadromy towards the north of its distribution area (McDowall 1988). The warm surface waters of lakes during the period of downstream migration have been compared to a "lid" through which fish do not migrate. Brown trout, *Salmo trutta*, transferred to southern areas have become sea-run only in those areas where sea temperatures are sufficiently cool, as in Tasmania and southern New Zealand (McDowall 1988). Smolts of steelhead trout, *O. mykiss*, subjected to

an increase in temperature from 6 to 13°C reduced behavioral downstream migration in a raceway (Smith 1985).

Inversely, Hoar (1953) showed that temperature increases changed the orientation of sockeye, chum, and coho salmon, *O. kisutch*, from actively swimming upstream to actively swimming downstream. A threshold temperature, or a combination of temperature increases and temperature levels, have been proposed to trigger migration in Atlantic salmon smolts (Solomon 1978; Jonsson and Ruud-Hansen 1985). In some river systems, temperature may account for as much as 90–95% of the yearly variation in date of smolt descent (Jonsson and Ruud-Hansen 1985). The dates at which different stocks migrate tend to coincide with the general climatic conditions over the north-south range of the species, with more southern, and hence warmer, populations migrating earlier than more northern ones. Atlantic salmon often start their seaward migration when the temperature rises to 5–10°C, regardless of the date (Smith 1985). Several stocks of sockeye smolts have been shown to migrate when lake surface temperatures are in the range of about 4–5°C, which coincides with ice breakup in northern areas and with the spring turnover of water layers in lakes (Smith 1985). In Atlantic salmon, migratory behavior was stimulated above a threshold temperature during the day but not at night (Greenstreet 1992*a*). This threshold increased as the average water temperature rose (Greenstreet 1992*a*) and was higher in mature than in immature smolts (Greenstreet 1992*b*).

The physiological mechanisms involved are only partly understood. High temperatures may reduce the ability of maturing adults to undertake upstream migration, since the maximum sustainable swimming speed of fish decreases sharply as water temperature rises above a thermal optimum (Hammer 1995). High temperatures may inhibit the development of seawater tolerance or accelerate its reversal (Adams et al. 1975; Zaugg and McLain 1976) and this may in some way inhibit downstream migration. A number of behavioral responses to temperature have been recorded in salmonids, including avoidance, hiding, social aggregating, loss of territoriality, and reduced feeding (Smith 1985). At least two of these changes, social aggregation and loss of territoriality, may favor migratory behavior. Finally, temperature may control the rate of physiological response to photoperiod such that the effects are apparent sooner at elevated temperatures (Wedemeyer et al. 1980).

1.1.1.3. Rainfall, river flow, and water turbidity

Rainfall, increased river flow, and water turbidity, have been shown to stimulate downstream migration of smolts, immature autumn parr, and mature male parr, as shown by enumeration of downstream migrants in rivers (Solomon 1978; Yamauchi et al. 1985; Youngson et al. 1983). Hatchery-reared Atlantic salmon smolts similarly showed higher rate of migration from seasonal holding ponds as water flow increased (Rottiers and Redell 1993) or during heavy rain (Greenstreet 1992*a*). Water flow during the last few months of life in the hatchery and relative water levels in the river at release have been identified as two of the three main determinants for adult return in hatchery-reared Atlantic salmon released as smolts (Hosmer et al. 1979; Hvidsten and Hansen 1988). Thus, water flow seems to have both acute and long-term effects on migration. Coho salmon, rainbow trout, *O. mykiss*, and brown trout have been shown to move upstream and spawn in response to freshets, whereas rainbow trout and chinook salmon, *O. tshawytscha*, tended to enter rivers when the barometric pressure was falling, anticipating rainfall (Smith 1985).

The selective advantage of these factors for downstream migrants may be to reduce predation by increasing migration velocity, increasing water depth, and decreasing visibility through the water surface (Hvidsten and Hansen 1988; Berggren and Filardo 1993). Under

controlled laboratory conditions, turbidity had a marked effect in reducing the predator avoidance behavior of juvenile chinook salmon (Gregory 1993). Increased migration velocity may also reduce the risk of desmoltification (cf. section 3.2.2) during migration. During upstream migration of adult salmonids, rainfall may provide an adequate water depth for entering small tributaries (Smith 1985).

Several possible mechanisms exist. Increased water flow and turbidity could decrease the ability of fish to maintain position in the stream, thus being passively displaced downstream (Smith 1982). In contrast with parr, smolts do not use their pectoral fins to anchor themselves to the bottom (Peake and McKinley 1998). High water flow may surpass the swimming ability of the smolt and high turbidity may further reduce their ability to maintain a visual fix. There is some evidence, however, that active behavioral changes are involved. Wild smolts show a high swimming performance, some smolts being able to swim indefinitely against flows up to eight time their body length per second (Peake and McKinley 1998). Others, however, appear unwilling to resist stream velocities higher than twice their own body length per second and are likely to turn head downstream and swim with the current above this critical level (Thorpe 1988). Stocks of inlet sockeye fry have also been shown to respond to increased water flow by orienting downstream, switching from positive to negative rheotaxis as velocity increases (see Smith 1985). This allows the fry to move downstream to the nursery lake when in the river but to resist against being swept out of the lake as they approach lake outlets (Smith 1985). Increased water flow may also elicit schooling behavior and decreased aggression, as shown in hatchery-reared salmonids (Jobling 1994). Such behavioral changes may in turn favor migration through social facilitation. Turbidity has also been shown to affect swimming behavior. Juvenile chinook salmon tended to leave the bottom when water became turbid (Gregory 1993). If a similar response exists in wild smolts, they would be swept away easier or join other migrants under conditions of turbidity. Finally, rainfall may induce changes in temperature or chemical composition of water that in turn induce migration. Heavy rainfall caused rapid migration of Atlantic salmon smolts down a release ladder without the flow being affected (Greenstreet 1992*a*). Natural freshets were more efficient in stimulating river entry of Atlantic salmon than artificial freshets caused by releasing water from dams (Smith 1985). The low barometric pressure during natural freshets could also stimulate downstream migration.

Increased water flow, changing water temperature, and chemical composition may all increase plasma thyroxine concentration (Youngson et al. 1986; Youngson and Mc Lay 1989; Youngson and Webb 1992) that in turn may induce migration (cf. section 4.1.for details). Thyroxine also seems to be central in stimulating imprinting (Hasler and Scholz 1983; Morin et al. 1994) and acts in synergy with growth hormone in stimulating smoltification (Leloup and Lebel 1993). Exercise induced by high water velocity increases plasma levels of growth hormone in rainbow trout (Barrett and McKeown 1989). Therefore, changes in plasma thyroxine could mediate the acute effects of increased water flow on migration, while changes in plasma thyroxine and growth hormone could mediate the long-term effects of water flow on adult return (see above).

1.1.1.4. Moon cycles

Downstream migration of smolts, immature parr, mature male parr, or newly emerged fry is often associated with the new moon or the full moon (Mason 1975; Youngson et al. 1983; Yamauchi et al. 1985). When hatchery-reared coho salmon were released on the new moon closest to the expected peak of plasma thyroxine, recoveries were approximately twice as high as the previous releases that were not lunar based (Nishioka et al. 1983).

Grau et al. (1981) underlined some of the advantages of using moon cycles as external zeitgeber for downstream migration. First, the dark nights of a new moon would reduce the vulnerability of small fish to predators. Second, the period length of the moon cycle is short enough to be used at all latitudes, despite migratory readiness being reached later in northern stocks than in southern ones.

The sensory mechanisms involved are uncertain. Changes in illumination, earth-moon-sun gravitational forces, or geophysical forces may be involved (Leatherland et al. 1992). Peaks of plasma thyroxine concentration synchronized with the moon cycle have been reported frequently (Hoar 1988). It is, however, uncertain how migration and peak thyroxine are related (Leatherland et al. 1992).

1.1.2. Biological factors

1.1.2.1. Size

A species specific threshold size has to be reached before anadromous salmonids migrate to the sea (McCormick 1994). Tipping et al. (1995) observed that when smolts of steelhead trout were released in a river, the percentage of emigration would increase with size until the fish reached 190 mm body length, above which greater size conferred no emigration advantage. Variations in this threshold size may exist within species, according to latitude (L'Abée-Lund et al. 1989), growth rate (Økland et al. 1993), sex, and early maturation (Fängstam et al. 1993). In conditions of low food supply, such as in overcrowded northern lakes, only a small fraction of the population may reach a sufficient size and become anadromous (Svenning et al. 1992).

Some species undergo a smoltification process when they reach the threshold size. In these smoltifying species, smoltification-associated behavioral changes may trigger downstream migration (Hoar 1988). Indeed, larger fish and repeat migrants that smoltify faster or earlier than smaller ones (Wedemeyer et al. 1980; Rydevik et al. 1989; Bœuf 1993) tend to migrate earlier in the season (Johnson 1980; Ewing et al. 1984*a*; Black and Dempson 1986; Bohlin et al. 1993; Nordeng 1977; Näslund 1990). In species considered to be nonsmoltifying, such as the brook trout, *Salvelinus fontinalis* (McCormick et al. 1985), the mechanisms linking size and downstream migration are unknown.

1.1.2.2. Condition factor

The condition factor (CF) is an estimate of the "well-being" or weight–length relation of the fish and is most commonly defined as (Anderson and Gutreuter 1983):

$$CF = (\text{body weight (g)}) \cdot (\text{fork length (cm)})^{-3} \cdot 100$$

Downstream-migrating wild salmonids have low condition factor (Rodgers et al. 1987) and hatchery-reared salmonids released into a river or allowed to migrate from hatchery raceways preferentially migrate when their CF is below some threshold level (Ewing et al. 1984*b*; Ewing et al. 1994; Tipping et al. 1995). During smoltification, an acute and temporary decrease in condition factor occurs around the time of migration, which corresponds to decreased weight and increased length (Bœuf 1993; Young et al. 1995). Smolting in heated water seems to prevent this decrease in CF (Soivio et al. 1988), which may be associated with the lower migration tendency observed in warm areas (McDowall 1988). From an evolutionary point of view, it seems appropriate that such a drastic physiological change as sudden growth in length is closely associated with the fish's ability to reach feeding habitats, in order to replenish the suddenly depleted energy stores.

1.1.2.3. Growth rate

Observations of size and age at smolting of wild Atlantic salmon and brown trout indicate that fast-growing fish smoltify and migrate at a smaller size than slow-growing ones (L'Abée-Lund et al. 1989; Økland et al. 1993). Økland et al. (1993) suggested that fast-growing parr have a higher metabolic rate, leading to the ability to osmoregulate in seawater at a smaller size and to a greater need for enhanced food supply. These fish may therefore be constrained earlier by the limited food resources in freshwater, which may, in some way, stimulate migration. In some populations, however, high growth rate is associated with early sexual maturation, which tends to inhibit or delay smoltification and migration (Thorpe 1987; Saunders et al. 1982). Water temperature and photoperiod, which both affect growth opportunity of parr, have been shown to be major determinants of the age at migration of Atlantic salmon and brown trout smolts (Metcalfe and Thorpe 1990; L'Abée-Lund et al. 1989). In a study of 182 Atlantic salmon populations of Canada and Europe, more than 80% of the variation in age at smolting could be explained by an index of growth opportunity that took into account both water temperature and daylength (Metcalfe and Thorpe 1990).

1.1.2.4. Age

In most species of anadromous salmonids, age at migration is highly variable and seems to depend on growth rate and minimal size for migration. There is some evidence, however, that in some stocks, age may be a major factor in determining onset of seaward migration. A river system in Alaska in which all fish had been killed by rotenone was stocked with sockeye salmon from a nearby river. Although the fish grew 4–5 times faster in the foster river than in the original river, they kept on migrating at the same age, independently of size (Smith 1985). Finstad and Heggberget (1993) also reported that in five Norwegian water-courses, the average age of first-time migrating Arctic char, *Salvelinus alpinus*, was consistently 5 years whereas mean size varied from 166 mm in the most northern river to 220 mm in the four other watercourses. Rich and Holmes (1928, cited in Randall et al. 1987) found that the progeny of chinook salmon migrated at the same freshwater age as their parents had, even though incubated in a hatchery and then transplanted to rearing streams where the resident population typically migrated at a different age. A genetic basis for the length of the freshwater residence time in "ocean-type" and "river-type" chinook salmon, a few months and 1 year or more, respectively, has recently been demonstrated (Clarke et al. 1994).

1.1.2.5. Sex and sexual maturation

Upstream migration and sexual maturation are closely associated in most Pacific and Atlantic salmon. A few stocks of steelhead trout and Atlantic salmon have nevertheless been described, in which adults return to freshwater one year before they spawn (Saunders 1981). Apart from these rare stocks, Atlantic salmon typically spend 1 or more years at sea but invariably return to freshwater as the gonads develop. Sexual maturation is associated with the appearance of electrophysiological and behavioral responses to specific odorants, including sex steroids (Moore and Scott 1991; Moore and Scott 1992) and odors imprinted during smoltification (Hasler and Scholz 1983), which guide the fish towards spawning grounds. Males and females may have slightly different ascending periods (Buck and Youngson 1982; Berg and Berg 1993).

When sexual maturation occurs in freshwater, it has an inhibitory effect on migration. During autumn migration of Atlantic salmon parr, mature individuals do not migrate as readily as immature parr and when they do, migration is delayed as compared to immature

juveniles (Buck and Youngson 1982; Fängstam et al. 1993). Buck and Youngson (1982) suggested that the presence of sexually mature adults temporarily inhibits downstream migration, enabling the mature parr to participate in reproduction. The mechanism involved is unknown but one could speculate that maturing parr become attracted to home spawning grounds in the same manner as maturing adults. Whereas such attraction induces migration towards the spawning grounds in adults, it prevents the parr from leaving them, inhibiting downstream migration. Both imprinted odors and sex steroids could be involved. Moore and Scott (Moore and Scott 1991; Moore and Scott 1992) showed that precocious male Atlantic salmon responded to specific sex steroids at a specific time period or after preexposure to urine of ovulated females. Sexual maturation could also inhibit downstream migration by inhibiting smoltification. A number of experiments suggest that sexual maturation and smolting are mutually inhibitory processes in salmonids, although high growth may allow both to occur (Thorpe 1987; Saunders et al. 1994).

1.1.2.6. Social facilitation

Seaward migration of salmonids typically occurs by mass movements. Groups of fish traveling together reduce metabolic costs by using turbulence (Weihs 1984). Moreover, schooling increases the chances of finding food, supplies some protection against predation, and decreases the error in orientation direction (Smith 1985). In Baltic salmon, *Salmo salar*, the number of smolts migrating together tended to increase during the migration season, forming schools of up to 180 smolts (Fängstam et al. 1993). Survival of released hatchery-reared Atlantic salmon smolts increased with the size of the school of migrating wild smolts into which they were released (Hvidsten and Johnsen 1993).

Mass movements could result from a common response to some stimulus such as loss of ice cover, a threshold photoperiod, temperature, or lunar phase. Alternatively, migrating individuals could stimulate other smolts in their vicinity to migrate. There is a strong tendency for grouping animals such as schooling fish to synchronize their activity (Smith 1985). Release experiments showed that hatchery-reared rainbow trout, 9–12 months old, migrated more quickly and completely out of an experimental stream, when released in large groups rather than in small ones (Smith 1985). In the river Orkla, Norway, Atlantic salmon smolts from upstream areas appear to stimulate the descent of smolts situated further downstream (Hvidsten et al. 1995). Individual Atlantic salmon smolts were more influenced by abiotic conditions than were schools (Bakshanskiy et al. 1987), which suggests that social interaction may override environmental stimuli. Daytime migration of smolts seems to increase as the number of migrating fish increases, either following the release of hatchery-reared smolts or at the end of the migration season (Hansen and Jonsson 1985; Fängstam et al. 1993). Therefore, social facilitation may induce migration of fish possibly at slightly different physiological states. Individuals leading the schools of Baltic salmon smolts were equally females, previously immature males, or previously mature males, of all sizes (Fängstam et al. 1993).

1.1.2.7. Endogenous rhythms

The existence of endogenous rhythms synchronized by environmental factors is often put forward to explain the seasonal occurrence of the changes associated with smoltification (Hoar 1988). Such rhythms are defined as being capable of self-sustained oscillations, which means that changes should occur rhythmically in the absence of environmental cues (Ali et al. 1992). This has apparently not been documented for migratory behavior of salmonids.

Eriksson and Lundqvist (1982) reported that Baltic salmon kept under constant photoperiod (LD12:12) and temperature (11°C) for 14 months smoltified twice at about a 10-month interval. The studied parameters that were condition factor, silvering, and fin blackening, tended to become out of phase. Thus, in this stock, seasonal changes in photoperiod and temperature probably synchronized and delayed the circannual occurrence of decreased condition factor and smolt-like appearance. Migratory tendency was not studied.

In an earlier study by Wagner (1974), the effect of photoperiod and temperature on migration was analyzed by exposing steelhead trout fry to different combinations of photoperiod and temperature regimens. The duration of the study was too short to draw any conclusion about a putative endogenous rhythm. However, Wagner (1974) observed that some of the fish kept in constant darkness and temperature developed migratory behavior, indicating that this behavioral change may occur in the absence of environmental cues. Migratory behavior occurred at a later date and when the fish were larger, as compared to controls. Therefore, changes in photoperiod and temperature or the presence of light apparently advanced the development of migratory behavior in this stock. Migration tendency was associated with a decrease in condition factor, silvering, and increased thyroid activity, i.e., part of a larger smoltification process. Only a few individuals migrated as compared to controls. This suggests that migratory behavior could be dependent upon the synchronization of several of the changes associated with smoltification, which in most individuals would require a rhythmically changing environment. Under constant conditions, some critical aspects may become out of phase, as was shown for condition factor and silvering in Baltic salmon (Eriksson and Lundqvist 1982), and this may impede migration. The highest migration tendency was obtained when both temperature and photoperiod were changing and were synchronized (Wagner 1974), indicating that several synchronized, rhythmic environmental cues could reinforce their effect on smoltification and migration.

Feeding is a potent entrainer of circadian rhythms in fish (Spieler 1992) and may have major effects in entraining circannual rhythms as well. It has been shown that seasonal changes in appetite in juvenile Atlantic salmon are matched with seasonal changes in food availability in the wild (Simpson and Thorpe 1997). A natural rhythm in food availability could therefore potentially entrain some aspects of smoltification. This may explain why smoltification and migration in the wild are usually better synchronized among individuals than they are under hatchery practice. In hatchery-reared salmonids, the addition of appropriate rythmic cues in food availability or quality to rhythms in temperature and photoperiod may improve the synchronization of smoltification between individuals and the development and time-relation between different aspects of smoltification within individuals, leading perhaps to a more appropriate migratory behavior.

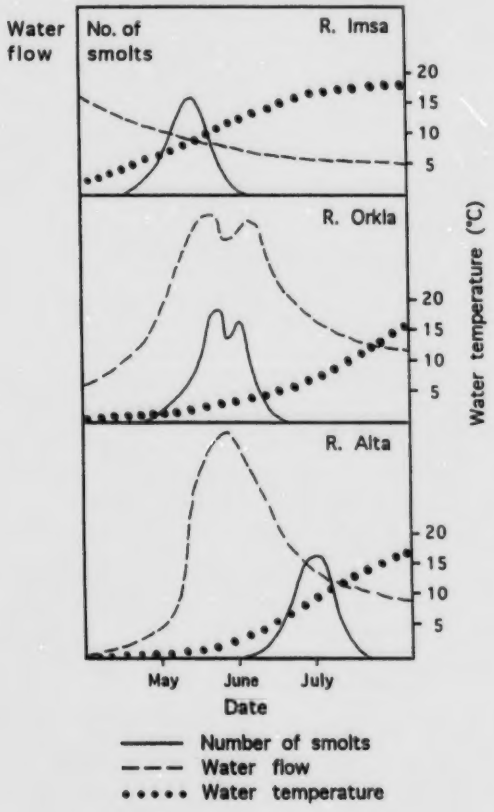
1.1.3. Relative significance of the different factors

The number of factors suggested to influence migratory behavior and the number of apparently conflicting results concerning their influence clearly demonstrate the complexity of the regulation of migration in anadromous salmonids. Each stock seems to respond to a specific selection of stimuli, possibly ranged in a specific hierarchy. When the dominant stimulus is absent at a certain time or physiological stage, backup systems may be used. Once the fish have reached a given physiological state, they could use the first-occurring stimulus among a number of environmental changes to ensure mass migration. It seems that photoperiod, temperature, and growth most often regulate the development of migratory

readiness, whereas moon cycles, light intensity, water discharge, or temperature changes are responsible for triggering downstream migration. The relative importance of all these factors seems to vary greatly among species, places, time of the year, and successive years.

This complexity must be seen as a major factor for the success of salmonids in colonizing a great variety of biotopes. The physiological state at which the fish become responsive to triggering factors and the nature of these factors must be adapted to local conditions. Smoltifying species of long river systems should, for example, respond to triggering factors at an earlier stage of smoltification than those from short rivers, in order to reach seawater at maximal seawater adaptability. Fish from different river systems should respond to different proximate factors in order to reach sea at an optimal period (ultimate factor). The time for onset of migration in relation to date, river temperature, and water flow in three streams along the Norwegian coast are illustrated in Fig. 1 (from Heggberget et al. 1993). In the River Imsa (59°N), smolts migrate early, at low or decreasing water flow, and at high

Fig. 1. Date, river flow, and river temperature during migration of Atlantic salmon smolts in three Norwegian rivers. In all cases, smolt descent was correlated with a sea temperature of 7–9°C. (Reprinted from Heggberget et al. 1993. Interactions between wild and cultured Atlantic salmon: a review of the Norwegian experience. Fish. Res. 18: 123–146. Copyright (1993), with permission from Elsevier Science.)



(8–10°C) and increasing water temperature. In the River Orkla (64°N), there is a clear seasonal variation in water flow which closely coincides with smolt migration, although temperature is still low (3–6°C). In the River Alta (70°N), there also is a clear peak in water flow during spring, but the fish migrate about 1 month later, as temperature is high (8–10°C) and increasing. In all cases, the smolts reach seawater when sea temperature is about 7–9°C. Thus, each stock seems to have developed a specific proximate trigger system for migration adapted to local conditions (Heggberget et al. 1993).

In northern areas, migration has to be precisely adapted to the short summer period during which food availability, temperature, and ice-conditions are favorable. Anadromous brook trout show highly synchronous migrations in northern latitudes, whereas southern populations show more variation in timing and duration of seaward migration (McCormick et al. 1985), supposedly adapted to some other local conditions. Increased water flow has the advantage of increasing the rapidity of migration and may be used to trigger mass migration in river systems showing clear variations in this parameter. Atlantic salmon are highly responsive to water velocity for onset of migration in the Girnock Burn but not in the Imsa River, which differs crucially from the Girnock Burn in showing a more stable seasonal discharge pattern (Youngson and Simpson 1984; Jonsson and Ruud-Hansen 1985). Rainfall may be necessary for migrating through small rivers. Coho salmon migrate upstream in response to precipitation, whereas coho salmon respond to falling barometric pressure, most often anticipating rainfall. Smith (1985) proposed that such behavior is adapted to the spawning habitats of the two species. Coho salmon usually spawn in small streams, where actual precipitation facilitates entry. In contrast, chinooks usually enter large rivers and additional rainfall may therefore not be necessary before the fish reach the headwaters.

As Northcote (1984) concluded, "The more closely we look at the detailed aspects of migratory behavior in riverine fish populations, the more evidence we uncover for marked local variation of a highly adaptive nature. Much of this variation seems to have a genetic basis so that fluctuating selective pressures even within habitats may operate quickly to shift responses in relatively few generations."

1.2. Motor activity during migration

1.2.1. Swimming pattern

1.2.1.1. During downstream migration

Downstream migration can result from basically three swimming patterns. Fish may actively swim downstream headfirst, passively drift downstream, or actively swim upstream more slowly than the river and thus be carried downstream tail first. There is evidence that all options may be used by smolts, depending on species, stocks, water flow, and time of the day. Atlantic salmon (Thorpe 1982) and Pacific salmon (Smith 1982) smolts have been shown to migrate downstream about 1/4–1/3 the velocity of the river, which suggests that migration is intermittent or that the fish actively swim upstream for periods. Acoustic tracking studies have shown that downstream progress is intermittent and that the step-lengths may be only a few hundred metres at a time (Thorpe 1988). Migration occurs mainly during dark periods (Northcote 1984; Thorpe et al. 1988). Pacific salmon have been shown to migrate at least 12–18 h per day, partly swimming actively upstream more slowly than the river (Smith 1982). Passive migration was observed in Atlantic salmon smolts for about one fourth of the day (Thorpe 1982). Active swimming downstream was observed during 10% of total time in Baltic salmon (Fängstam 1993). Sockeye smolts have been observed to

swim actively downstream mainly during the night, interrupting migration during the day (Smith 1985).

Some smolts appear unwilling to resist stream velocities higher than twice their own body length per second and are likely to turn head downstream and swim with the current above this critical level (Thorpe 1988). Others have been shown to swim for long periods (>200 min) against flows up to eight body lengths per second (Peake and McKinley 1998). It has been suggested that in turbulent water, smolts may orient upstream and drift downstream tail first (Hasler and Scholz 1983). Smith (1982) proposed that an advantage of such behavior was to avoid obstructions easier by being in position to rapidly spurt ahead, upstream. A genetically determined threshold of water velocity at which fish switch between swimming upstream and downstream has been evidenced in sockeye fry (Smith 1985). In migrating smolts, water temperature, visibility, and characteristics of the river also probably affect swimming pattern.

The swimming pattern during downstream migration must be adapted to the freshwater habitat of each species and stock. Whereas sockeye salmon and most Arctic char utilize lakes as nursery areas, Atlantic salmon utilize rivers, while coho, chinook salmon, and steelhead trout may utilize both (Smith 1985). Whereas sockeye salmon migrate actively through lakes, Atlantic salmon released experimentally upstream from lakes or impoundments move very slowly through these, as slow as surface water movements (Thorpe 1988). However, some Atlantic salmon stocks normally cross lakes situated downstream from their nursery areas and there may well be local adaptations (Smith 1982; Hansen et al. 1984). Sockeye smolts, 8 cm long, must migrate through lakes that may have dimensions measured in tens of kilometres and in which the water currents are determined primarily by wind direction (Smith 1985).

The complexity of swimming patterns involved in downstream migration certainly represents an adaptive advantage. The passive downstream drift reduces the energetic cost of migration. Downstream migrating smolts may undertake long migrations at a small size. Energy stores in these fish are limited and must cover the demands associated with structural and biochemical changes necessary for seawater life. On the other hand, active swimming allows them to cross lakes or impoundments and may allow smolts to reach new habitats. Such an exploratory behavior may explain how a population of landlocked Arctic char has developed yearly migrations to summer feeding habitats situated upstream from the lake of residence (Näslund 1990). These fish live in an oligotrophic lake and feed during summer in a lake situated 5 km upstream from their lake of residence (Näslund 1990).

1.2.1.2. During upstream migration

Upstream migration may occur as a result of active upstream movement, holding position, and occasionally downstream movement. Wild Atlantic salmon commonly move rapidly to precise areas close to the spawning grounds, then hold position for a long period (up to several months), and finally make a short upstream migration just before spawning (Heggberget et al. 1988; Smith and Laughton 1994). Long migrations may include several steps (Thorpe 1988). After artificial displacement or accidental overshooting upstream from the home areas, both Atlantic and Pacific salmon tend to move downstream in an attempt to find the home areas (Heggberget et al. 1988). "Back-tracking" is also observed in fish that have entered a wrong tributary at a stream junction to rectify their error (Hasler and Scholz 1983). A scarcity of orienting cues may increase the occurrence of downstream movement (Power and McCleave 1980).

The pattern of diel movements is quite variable and migration may occur at any time of day or night (Smith 1985). However, at some points of the river, such as waterfalls or fish ladders, the fish may prefer either light or dark hours and thus accumulate below the barrier until the appropriate time occurs (Smith 1985).

1.2.2. Swimming speed and physiological adjustments

1.2.2.1. Swimming speed

The swimming speed of fish may be classified into three major categories. Sustained swimming, called "cruising" in the case of migrating fish, can be maintained for long periods (>200 min) without resulting in muscular fatigue (Beamish 1978). Prolonged swimming can be maintained for a moderate period of time (20 s – 200 min) and ends in fatigue. And finally, burst swimming is high speed swimming for <20 s (Beamish 1978). It can be assumed that salmonids migrate mainly at a speed that can be maintained for weeks or months without resulting in muscular fatigue, i.e., cruising, interrupted by short periods of prolonged or burst swimming, for example, to avoid predators or negotiate natural barriers and rapid currents. These periods are probably followed by a recovery period at low swimming speed.

Minimal estimates of mean swimming speed may be obtained by releasing and recapturing tagged fish and assuming that they swim in a direct line between the two registration points. With such a method, sockeye salmon have been shown to travel at average minimum speeds ranging from 11.5 to 77.8 km per day in the ocean (Healey and Groot 1987). Assuming that these fish were 80 cm long (L), this represents minimum average speeds of 0.2–1.1 $L \cdot s^{-1}$ (Healey and Groot 1987). Atlantic salmon have been shown to migrate more than 100 km a day when swimming with the current along the Norwegian coast and up to 60 km a day when swimming against the current (Hansen et al. 1993). No significant difference in the migratory speed of small ($L < 75$ cm) and large (>75 cm) fish was found in that study (Hansen et al. 1993).

Ultrasonic tagging experiments may provide more accurate information. Such observation of sockeye salmon in the ocean showed that fish of an average length of 66.3 cm had an average speed of 66.7 $cm \cdot s^{-1}$, very close to 1 $L \cdot s^{-1}$, or 58 km per day (Videler 1993). Atlantic salmon have been reported to migrate as fast in freshwater rivers as in the sea but to hold station for up to 14 days in the estuary, possibly for osmoregulatory adaptations (Beamish 1978). A positive correlation between fork length and migration velocity has been observed both in downstream migrating smolts (Ewing et al. 1984*a*) and upstream migrating adults (Baglinière et al. 1990; Baglinière et al. 1991). Expressed relative to body length (L), mean swimming speed reported in adult pacific salmon is generally between 0.5 $L \cdot s^{-1}$ and 2 $L \cdot s^{-1}$ in rivers or open water (Beamish 1978). Factors of variation include water currents, temperature, light conditions, or time of the day (Beamish 1978). In smolts, migration speed also depends on season and stage of migration. Zabel et al. (1998) showed that yearling chinook salmon migrated more rapidly later in the season. Moreover, they accelerated as they progressed, migrating faster in lower reaches of the river (Zabel et al. 1998).

Under laboratory conditions, a critical speed, which is presumed to reflect relatively closely the maximum aerobic capacity of the fish, can be determined (methods reviewed by Hammer 1995). Factors that influence this critical speed include genetics, size, dietary and carcass protein content, training, spawning, season and temperature, light, and water oxygen content (Hammer 1995). Larger fish swim faster than small ones when this speed is expressed as an absolute value (e.g., $cm \cdot s^{-1}$) but slower when expressed relative to the body

length of the fish (e.g., $L \cdot s^{-1}$). This is despite the fact that large fish have a higher relative amount of muscle mass than small fish (65% of the body mass in a 1000 g salmon and 35% in a 10 g salmon) (Videler 1993). At temperatures of 5–20°C, the critical speed of an Arctic char of 32 cm was close to 2 $L \cdot s^{-1}$. As temperature increased above 20°C, the critical speed sharply decreased (Hammer 1995). In 10-cm-long wild coho salmon smolts, the critical speed was approximately 5.5 $L \cdot s^{-1}$ (Brauner et al. 1994).

During burst swimming, recorded speed is typically 8–10 $L \cdot s^{-1}$ in salmonids when measured over 1–5 s, and 2–8 $L \cdot s^{-1}$ when measured for 10–15 s (Beamish 1978).

1.2.2.2. Cardiovascular adjustments

Cardiovascular adjustments are necessary to improve oxygenation of muscle tissues during exercise or during recovery from strenuous exercise. Oxygen consumption increased sixfold in rainbow trout swimming at 81–91% of critical swimming speed (Jones and Randall 1978). Following exercise, excess oxygen is needed to rebuild stores of oxygen, ATP, and creatin phosphate ("alactacid oxygen debt") and to reoxidize lactate to glycogen ("lactacid oxygen debt"). While the first may require only a period of few minutes, the reoxidation of lactate is slower and may require several hours (Jobling 1994).

During sustained or prolonged swimming, cardiac output increases and blood flow is redistributed to the swimming muscles at the expense of the viscera (Farrell 1993). Increased cardiac output in teleosts is achieved through adjustment of stroke volume rather than heart rate (Jones and Randall 1978). In rainbow trout swimming at critical speed, cardiac output was threefold the value at rest (Farrell 1993). Heart beat frequency increased by one-third, while stroke volume doubled. Total blood flow increased more in red muscle than in white. Whereas total blood flow to red muscle was lower than to white muscle in resting rainbow trout, it became higher during prolonged or exhaustive exercise, total blood flow to lateral red muscle more than quadrupling (Farrell 1993). During burst exercise, however, heart beat frequency, cardiac output, and arterial blood pressure decreased (Farrell 1993).

In resting rainbow trout, only two-thirds of the gill lamellae are normally perfused at one time. During exercise, lamellar recruitment occurs. This may be due to catecholamine mediated vasodilatation of afferent and efferent lamellar arterioles and (or) to an increase in input pressure above the critical opening pressure of afferent lamellar arterioles (Farrell 1993).

1.2.2.3. Respiratory adjustments

Ventilation increases during exercise. At moderate speed, the rate and amplitude of ventilatory movements increase. At speeds above 50–80 $cm \cdot s^{-1}$, however, most salmonid fish stop ventilatory movements, relying entirely on the relative movement of surrounding water for ventilation (ram ventilation) (Jones and Randall 1978).

Blood oxygen transport capacity also increases during exercise. Red blood cells are mobilized from the spleen as a result of α-adrenergic induced splenic contraction (Perry and McDonald 1993). An additional hemoconcentration may occur due to fluid shifts from extracellular to intracellular or external compartments. Hemoconcentration tends to increase blood viscosity, increasing cardiac work. There is, however, some evidence that adrenergic induced swelling of the red blood cells may limit the increase in blood viscosity (Perry and McDonald 1993).

During exhaustive exercise, lactacidosis may occur (see 1.2.2.5). In teleosts, intracellular acidosis not only decreases haemoglobin affinity for oxygen (Bohr effect) but also its maximal binding capacity (Root effect) (Perry and McDonald 1993). However,

salmonids are capable of minimizing or totally preventing changes in intracellular pH of red blood cells during extracellular acidosis. This is achieved by a β-adrenergic activation of the Na^+/H^+ exchange through the red blood cell membrane, following acute release of catecholamines, which probably occurs in response to blood acidosis and (or) hypoxia. While proton efflux limits intracellular acidosis, sodium influx causes red blood cell swelling due to osmotic entry of water. In addition, a depletion of ATP occurs, possibly due to the additional energetic demands of the Na^+/K^+ pump as the sodium level in the cell rises. ATP is the principal intracellular nucleoside triphosphate in salmonids and a decrease in ATP causes an increase in haemoglobin–oxygen binding affinity (Perry and McDonald 1993).

Within teleosts, salmonid red blood cells display the greatest responsiveness to catecholamines (Perry and McDonald 1993). Moreover, during spring and summer, production of erythrocytes by Atlantic salmon is higher than in winter and the β-adrenergic responsiveness of trout red blood cells is enhanced. This contributes to increased blood oxygen transport capacity during the migratory periods (Jensen et al. 1993).

1.2.2.4. Osmo-ionoregulatory consequences

Increased lamellar recruitment in the gills increases the passive movements of water and ions across the gill epithelium. The increase in water influx is compensated for by an increase in urine production (Jones and Randall 1978). Changes in plasma ion composition and ion exchange over the gill epithelium are minimal at moderate swimming speeds, whereas during exhaustive exercise, profound ionic and osmotic disturbances may occur (Jobling 1994). Special adaptational features apparently allow the most active salmonids, such as rainbow trout, to experience lower ion losses for a given increase in oxygen transfer than the less active lake trout, *Salvelinus namaycush* (Perry and McDonald 1993).

Anaerobic muscular activity leads, moreover, to the intracellular accumulation of lactate, which causes extracellular water to be transferred to the intracellular compartment (Jobling 1994).

1.2.2.5. Acid–base consequences and adjustments

Migrating salmonids are probably in acid–base balance most of the time. They may swim at speeds of 2–5 $L \cdot s^{-1}$ without needing anaerobic metabolism (Jobling 1994). Moreover, they may use anaerobic metabolism without experiencing any acidosis. In rainbow trout, white muscles were involved above 80% of the critical speed (Hammer 1995). Until about 92% of the critical speed was reached, however, arterial blood pH and blood lactate concentration remained stable, suggesting that lactate production was balanced by its clearance in the muscles, the liver, and the gills (Hammer 1995). Alternatively, white muscles may show some aerobic capacity. The metabolism of white muscle in maturing rainbow trout has been shown to become increasingly aerobic, with an increased capacity for fatty acid utilization (Kiessling et al. 1995). White muscle tissue contains large amounts of fat, stored in adipose cells dispersed among muscle cells (Sheridan 1989). Whether circulatory adaptations occur in maturing salmonids, providing the white muscle with enhanced blood supply to meet its increased aerobic capacity, has apparently not been studied.

Prolonged exercise resulting in acid–base disturbances probably occurs in salmonids as they try to migrate upstream through fast-flowing parts of the river or as they try to avoid large obstructions, such as traps across the river. In rainbow trout, exhaustive exercise has been shown to result in a mixed metabolic and respiratory acidosis (Heisler 1984; Heisler 1993). The respiratory part of the acidosis is due to the slow elimination of excess carbon dioxide through the gills. Plasma P_{CO_2} may typically increase from 0.27 to 0.53 kPa.

Normalization of plasma pH occurs mainly by the transfer of hydrogen ions to the ambient water. Lactate is never released into the environment and the normalization of plasma lactate concentration may require much more time. In rainbow trout, maximal disturbance of acid–base status and lactate concentration in plasma typically occur 1 h and 2–8 h, respectively, following exhaustive exercise (Heisler 1984; Heisler 1993). In muscle tissue, normalization of lactate and glycogen levels may occur within 30 min in red muscle but may require more than 5–8 h in white muscle (Woodhead 1975).

Mature salmon may recover faster than juveniles following intense exercise. Whereas a 15-min period of severe exercise required 24 h for complete normalization of acid–base status in young trout, adults of coho salmon and steelhead trout recovered within 2–3 h (Woodhead 1975).

1.3. Metabolic aspects of migration

As stated by Woodhead (1975): "A successful migration depends not only upon the appropriate behavioral responses to environmental stimuli, but equally upon closely regulated metabolic changes which enable the fish to mobilize sufficient energy reserves to sustain a movement which may be both prolonged and extensive — migration is as much a matter of metabolism as of behavior."

1.3.1. Energy requirements

During river migration, energy is needed for body maintenance, swimming activity, adaptation or preadaptation to salinity changes, and in the case of maturing adults, for gonad growth (Woodhead 1975). To understand how these needs may be covered during several months in fish that are generally fasting, one must remember that fish are poikilotherms and thus "a lot cheaper to run than homeothermic mammals. For comparison, the highest active metabolic rate of a 2-kg sockeye salmon equals the basal metabolic rate of a rabbit of the same weight" (Videler 1993). After onset of anorexia, maturing female Atlantic salmon, actively swimming around a 5-m seawater tank, lost weight at a rate of only 0.1% per day (Kadri et al. 1995).

1.3.1.1. Cost of swimming

According to Jobling (1994), the rate of oxygen consumption of an actively swimming fish is typically 3–7 times higher than oxygen consumption at rest and can reach 10–15 times the resting level of fish swimming at high speed. However, experiments performed with hatchery-reared salmonids have shown that forcing fish to swim at moderate speeds may decrease energy expenditure and increase growth rate (Hammer 1995; Adams et al. 1995). This may be due to the fact that fish in groups are rarely resting but rather show spontaneous activity including turns, fin movements, sudden velocity breaks, and agonistic behavior. During sustained swimming at moderate or high speed, fish become less aggressive and stop most of these spontaneous movements.

Migrating salmonids seem to take advantage of energy saving behavior because the theoretical energy requirements for moving a streamlined rigid body of the same length and surface area as a salmon is higher than the actual energy use (Smith 1985). "Two-phase periodic locomotion," which is the succession of an active acceleration followed by a period of coasting with no propulsive movements, produces large energy savings for sustained swimming over a long distance, as well as for anaerobic bursts of swimming (Weihs 1984). Swimming at a depth of at least three times the width of the fish reduces the drag to a

minimal value. A salmonid swimming at the surface would loose 80% of its swimming energy by generating waves (Videler 1993). Schooling behavior, which is largely used by smolts during seaward migration, reduces energetic costs by taking advantage of turbulence movements (Weihs 1984).

A theoretical optimal swimming speed for fish, leading to a minimal amount of work per traveled metre (see Videler 1993), has been proposed. According to Weihs (1984), for fish between 0.3 and 0.7 m in length, the optimal swimming speed ($m \cdot s^{-1}$) is given by the formula $0.5 \cdot L^{0.43}$, where L is the fish length in metres. According to Videler (1993), a relative optimum speed expressed as $L \cdot s^{-1}$ is given by the formula $1.1 \cdot M^{-0.14}$ and the energy needed to transport a fish with a body mass of M (kg) over one body length at that speed is given by the formula $0.5 \cdot M^{0.93}$ (J).

1.3.1.2. Cost of ventilation, osmoregulation, and acid–base regulation

The cost of ventilation does not increase in proportion to oxygen consumption as a result of ram ventilation at higher speeds. In rainbow trout, the cost of ventilation increased from 10% to 15% of total oxygen consumption as the speed increased to about 30% of critical speed. Above this level, the fish changed to ram ventilation, resulting in the cost of ventilation suddenly dropping to 5% of total oxygen consumption (Hammer 1995). The relative cost of ventilation at high swimming speeds may thus be lower than at rest.

According to Jobling (1994), the normal cost of iono- and osmo-regulation is thought to be usually as little as 1–2% of total metabolism. Following periods of exhaustive exercise, however, the restoration of ionic and acid-base balance impose metabolic costs that make a large contribution to the maintenance of the high rates of oxygen consumption observed during the recovery period (Jobling 1994).

1.3.1.3. Cost of gonad growth

In Atlantic salmon and different species of Pacific salmon, ovaries may grow from 1–7% of body weight at river entry to 14–27% of body weight at the spawning grounds, while that of testis may increase from less than 1 to 3–6% of body weight (see Woodhead 1975). In Atlantic salmon migrating up the River Drammen, Norway, the energy content per unit tissue mass was about twice as high in ovaries than in testis, due to a higher lipid content in ovaries (Jonsson et al. 1997). Expressed as a percentage of body energy content, the energy content of ovaries increased from 3 to 29%, while that of testis increased from less than 1 to 3% (Jonsson et al. 1997). In anadromous brown trout from southern Norway, the energy contents of ovaries and testis prior to spawning were 34 and 3% of body energy content, respectively (Jonsson and Jonsson 1997). Energy expenditure on the growth of gonads has been estimated in sockeye salmon at about 10% of total energy reserve present at the beginning of migration in females and 0.5% in males (see Woodhead 1975).

1.3.2. Energy mobilization

Early studies concerning the energetic cost of migration and sources of energy used during migration of salmonids have been reviewed by Woodhead (1975). In short, the main sources for energy during migration are lipids and proteins. During the 1150-km-long upstream migration of Stuart sockeye salmon, body lipid content fell by approximately 95%, whereas body protein content fell by about 30% in males and 50% in females. The total energy expenditure during this 34-day-long journey was approximately 5900 kJ in males and 6900 kJ in females. Most of the fat is mobilized from muscle tissue. The relative amount of fat in wet muscle decreased from 16 to 2% in king salmon, *O. tshawytscha*, following a

1100-km-long migration. The amount of fat mobilized from other sources (liver, mesenteric fat, skin, bones) was estimated as 16% in males and 35% in females of the amount of fat mobilized from muscles. During the initial phases of their river migration, salmon maintain their body weight by the uptake of water as fats are withdrawn. During the later stages of withdrawal, body weight may fall markedly. A king salmon of 86 cm lost 52% of the wet weight of muscle during its migration up the Sacramento river and total solids in the remaining muscle fell from 26 to 7% of total body weight. Total protein content in plasma also fell markedly during upstream migration of sockeye salmon (Woodhead 1975).

More recently, the total energy loss due to migration and spawning was estimated in Atlantic salmon migrating up the River Drammen, in Norway (Jonsson et al. 1997). It was found to be 60–70% of the body reserves prior to upstream migration, similar in males and females, and higher in large salmon than in small salmon. As in earlier studies on Pacific salmon, the energy source was mainly muscular lipids (Jonsson et al. 1997).

Liver cells may be completely depleted of fat at the end of migration but their glycogen content may be similar to, or even higher than at river entry (Woodhead 1975). Muscle and liver glycogen are used mostly during periods of high muscular activity such as burst swimming that is performed mainly anaerobically (Hammer 1995). Under experimental conditions, the restoration of muscle glycogen in exercised fish is slow, particularly when the fish are fasting (Woodhead 1975). However, carbohydrate metabolism of migrating salmonids seems to be adapted to their increased energy demands and the rapid restoration of glycogen stores by increased gluconeogenetic capacity. Insulin levels progressively decline during the return migration of salmonids (Murza et al. 1991; Mommsen and Plisetskaya 1991), while plasma glucocorticoids are generally elevated (cf. section 4.3). The activity of neoglucogenetic enzymes in both liver and muscle tissue was shown to increase during upstream migration of pink salmon (Maksimovitch 1981). There is some evidence that amino acids are mobilized from white muscle and metabolized to glucose in liver and red muscle (Maksimovitch 1981). Migrating Atlantic and Pacific salmon maintain a high blood glucose concentration, despite low food intake (Woodhead 1975).

The mobilization of energy stores during upstream migration has important consequences on the body compartments of the fish (Talbot et al. 1986). The intracellular space decreases while the extracellular space expands, with a corresponding decrease in body potassium and increases in body sodium and chloride. In Atlantic salmon, the intracellular fluid volume was estimated as 77% of total body water in farmed seawater adults but only 50% of total body water in wild adults following migration and spawning (kelts). Extracellular fluid volume was estimated to be 15% of wet body weight in seawater adults and 41% of wet body weight in kelts (vs. 14% on average in freshwater teleosts) (Talbot et al. 1986).

During upstream migration, a major mobilization of carotenoids from flesh is observed in both sexes. Up to 99% of the orange pigment astaxanthin is transported from flesh to skin and ovaries, via plasma, where it is bound to high and very high density lipoproteins (Ando and Hatano 1988; Torrissen et al. 1989). In addition to providing precursor molecules to vitamin A, this mobilization could play some role in protecting lipids rich in polyunsaturated fatty acids against peroxidation (Torrissen et al. 1989).

1.3.3. Selective significance

The energy consumption and swimming capacity of migrating salmonids appear to be important factors limiting their distribution. Among diadromous fish, the greatest distances

migrated upstream are found in salmonids, which are also the largest fish. Among salmonids, the longest migration distances are covered by the chinook salmon, the largest of the salmon species (McDowall 1988). In some populations, the larger and older individuals may spawn on far upstream spawning grounds and (or) migrate through difficult and swiftly flowing rivers, whereas smaller ones spawn nearer the ocean (Schaffer and Elson 1975). Large fish are stronger and more persistent swimmers and have better reserves of energy to achieve the upstream migration. Therefore, they have a higher chance of reaching the spawning grounds and are more likely to leave progeny, i.e., difficult rivers select for larger fish (McDowall 1988). Kadri et al. (1995) have suggested that some stocks may have to reach a specific, genetically determined, threshold of energy stores and muscular mass at sea, before they undertake upstream migration. This specific threshold should be adapted to the length and difficulty of the migration and to the requirements of gonadal growth (Kadri et al. 1995).

This phenomenon has been studied in some details in the Arctic char by Kristoffersen (1995). He demonstrated that the barrier index of the river, combining river length and water discharge, is an important factor in determining the proportion of migrants versus freshwater residents in Norwegian populations of Arctic char. When rivers are longer than 4–7 km, the proportion of migrants is low. The best combination for favoring anadromy seems to be relatively high velocity in a short river. Kristoffersen (1994) suggested that high water velocity probably makes predation difficult for birds and mammals and results in a shorter stay in the river for descending first-time migrants, while in long rivers, this positive effect may be outweighed by the negative effect of the energy costs during upstream migration. The Arctic char is particularly susceptible to the costs of migration because they feed in the sea for a period of only 5–7 weeks (Finstad and Heggberget 1993; Berg and Berg 1993); therefore, first-time migrants ascend the river at a small size, typically 150 g weight and 25 cm long. Only a few mature after one migration (Nordeng 1983), so most fish must have sufficient energy left to survive for about 10 months without feeding and to undergo smoltification-associated changes prior to the next migration (Kristoffersen 1994). An adaptive life strategy, in which young Arctic char migrate only a short way and older fish migrate longer, may exist in long river systems. However, a specific size-dependent geographical segregation of the Arctic char populations has to my knowledge not been described.

Salmonids seem to be able to adapt rapidly to local conditions by choosing the life strategy which is the most advantageous. When migration to the feeding habitat does not bring sufficient growth advantage, anadromy gives place to residency. Within each species, there has been a selection of anadromous phenotypes in northern areas, where food supply in freshwater is restricted, and of resident phenotypes in southern areas, where the costs of migration may be higher than its benefits. Within a population, age-classes that no longer find any feeding advantage of migrating may become resident (Näslund 1990). Thus, the feeding state of the fish may be expected to be an important physiological cue regulating migration. For the latitudinal segregation of anadromous populations, however, related cues such as temperature or photoperiod could also be used.

1.4. Orientation

Salmonids show a high capacity for orientation both during downstream and upstream river migration. Sockeye smolts, 8 cm long, find their way through lakes that may have dimensions measured in tens of kilometres and in which the water currents are determined

primarily by wind direction (see Smith 1985). Adult salmonids generally home with great precision to their spawning grounds (Quinn 1993), which allows them to confine spawning to river systems of proven suitability for survival and to divide into stocks highly adapted to local conditions (Smith 1985).

Senses possibly used by fish during their migration have been reviewed by Smith (1985). The possible use of water currents, olfactory and visual cues, magnetic fields, and temperature cues for orientation of salmonids will be presented here shortly. As Smith underlines, studies concerned with the mechanisms of orientation face several limits. Several sensory mechanisms in fish are based on diffuse organs, such as thermoreception, tactile reception, and possibly compass-like magnetoreception (Kirschvink 1997). These can not easily be blocked or destroyed selectively, in contrast to olfaction (plugging or cauterizing the nares, sectioning the olfactory nerve) and vision (blinding); most knowledge, therefore, concerns the two last-mentioned senses. Moreover, many studies are based on the release of tagged fish and the registrations of the river in which these fish are trapped or fished. Such experiments may underestimate homing, since they may not allow salmonids that have entered the wrong tributary to return to the stream junction and choose the right stream, a strategy which appears to be common in the wild (Hasler and Scholz 1983; Smith 1985).

1.4.1. Stream direction and velocity

When exposed to water flow, fish may orient downstream, maximizing the rate of movement, orient upstream and try to remain stationary, or move upstream. The first case is referred to as negative rheotaxis, the two latter as positive rheotaxis (Smith 1985). The use of water current as a directional cue in river migration of anadromous salmonids seems obvious. Negative or positive rheotaxis at the appropriate season or developmental stage would serve as a reliable mechanism for achieving either downstream or upstream movement. Indeed, a change from positive to negative rheotactic behavior occurs during smoltification, while the reverse change occurs during desmoltification (Schmitz 1992; Lundqvist and Eriksson 1985). In sockeye salmon, the presence of a genetically determined threshold of water velocity at which some sockeye fry switch between positive and negative rheotaxis may guide these stocks through complex migratory routes (Smith 1985). Such orientation cues could also be used by smolts or adults.

The oriented response of fish to current is a response to visual or tactile stimuli. It may occur in the absence of water movement just by moving objects in the visual field of the fish (Smith 1985). In the wild, fish may respond to the sight of the bottom or shore moving past as the current carries them along. During dark hours, they may use tactile stimuli, such as touching the bottom or vegetation. Social interaction between the fish in a school may extend visual or tactile information (Smith 1985).

1.4.2. Olfaction

Fish possess acutely sensitive chemoreceptors. Salmon can differentiate between the specific odors of rivers (Hasler and Scholz 1983). Arctic char have also been shown to differentiate between odors from adults and juveniles and between odors from different stocks (Døving et al. 1974; Hasler and Scholz 1983). Moreover, chemical information is available throughout the diel cycle and is not affected by depth and turbidity to the same degree as vision. The persistence of chemicals in water can carry information over long distances, supposedly hundreds of kilometres (Smith 1985).

Evidence for the role of olfaction in orientation of upstream migrating salmonids has been reviewed by Hasler and Scholz (1983), Smith (1985), and Døving (1989). In short, both genetic and learning processes are thought to be responsible for recognition of the specific home odor. Strong imprinting to the composition of the home river probably occurs during smoltification and transplantation experiments suggest that a few days or even hours of exposure to the river odor are sufficient during the optimal period (Hasler and Scholz 1983). During sexual maturation, an increase in olfactory sensitivity to the imprinted odor occurs, accompanied by a specific behavioral response to it (Hasler and Scholz 1983). In the river, maturing salmonids seem to respond by positive rheotaxis in the presence of the imprinted scent and a negative rheotaxis in its absence. Thus, if a fish makes the wrong choice at a stream junction, the imprinted scent will no longer be present and the fish will swim downstream until encountering it again. This mechanism is deduced by tracking studies of coho or Atlantic salmon during spawning migration, as well as tank experiments of rheotropic behavior in response to odors (Hasler and Scholz 1983).

Towards the end of the spawning season, adults cease to respond to their imprinted odor or to home water and, instead, begin to respond strongly to odors of other salmon (Hasler and Scholz 1983). Studies with precocious male Atlantic salmon parr have shown that an olfactory response to specific sex steroids may occur only at a specific season or after exposure to the urine of ovulated females (Moore and Scott 1991; Moore and Scott 1992). This may be important as a general mechanism for attracting fish that have failed to home, to sites with other adults, thereby allowing completion of their life cycle. This may be of particular importance for "homeless" hatchery-reared salmon that have escaped from net-pens and may explain the later ascent of fish lacking the juvenile experience of local streams. Hatchery-reared Atlantic salmon released as smolts in the lower part of the river Imsa ascended the river significantly later than wild fish, whereas smolts released in the upper part of the river ascended as adults at the same time as the wild fish (Jonsson et al. 1994). Interestingly, in this study, water to the hatchery came from the upper part of the river; thus, all fish had some experience of the home water. Moreover, the River Imsa is short (1 km) and the two sites of release differed only by 900 m. Therefore, the lack of cues other than the olfactory ones may have caused the delayed ascent of adults with no juvenile experience of the real river. Alternatively, there may be highly site-specific odor variations in this river and the scent of the water in the hatchery may have been affected by pipes, tanks, food, treatments, high fish density, or other hatchery-related conditions.

Spawning areas of salmonids are often close to, but different from, nursery areas (Smith 1985). Tracking studies of wild Atlantic salmon have shown that they commonly move rapidly to precise areas close to the spawning grounds then hold position for a long period (up to several months) before making a short upstream migration prior to spawning (Heggberget et al. 1988; Smith and Laughton 1994). This two-step migration could be related to the change in olfactory response described in Hasler and Scholz (1983). The fish would first move towards the scent imprinted during smoltification then later towards conspecifics and appropriate spawning grounds. Sockeye, pink, and chum salmon, however, emigrate from their spawning stream almost immediately upon emergence from the gravel and may cover long distances to their nursery area. A precise homing to spawning areas could therefore depend on an early imprinting of the embryo or the alevin in the gravels (Brannon 1982), at least in some stocks. The olfactory epithelium of the embryo 3 weeks prior to hatching appeared to be as well-developed as that of the adult on the basis of light microscopy and there is evidence of olfactory learning during this period (Smith 1985). Early imprinting has been documented in Horsefly sockeye salmon, which returned to their

site of incubation 20 miles upstream from their site of release as fingerlings (Brannon 1982). In this case, the fish were able to track the odor of the incubation site once they arrived at the release site. On the basis of several studies, Brannon (1982) suggested that adults return to the trunk stream on the basis of an imprinting process that occurs after release and then return to their native incubation site whenever its scent can be detected at the site of release. Thus, in the wild, salmon would imprint on their natal stream before leaving the spawning site and later imprint on one or several subsequent qualitative changes. This conforms to the sequential learning hypothesis by Harden Jones (1968). Evidence is available that imprinting at least during the smolt stage is sequential (Brannon 1982). Such a sequential imprinting may explain how salmon find their way back to small tributaries, even when the home water is diluted on its way to the sea by large amounts of water from numerous other tributaries. It would seem appropriate that the strong imprinting period studied by Hasler and Scholz (1983) occurs when the salmon are in a part of the river or estuary which is large enough to provide a substantial amount of specific odor traces in the sea. This would enable the adults to trace the trunk river at far greater distances than if imprinting occurred only in the nursery area. Sutterlin et al. (1982) have shown that imprinting may occur even in seawater and this may be appropriate when the home river trunk is too small to give a far-reaching trace in the sea. When Atlantic salmon smolts from the River Imsa were released at sea, 40 km from the estuary, they returned as adults to the area of release but failed to return to the River Imsa and entered nearby rivers for spawning (Hansen et al. 1993).

If there are only one or a few imprinted scents, then the maintenance of positive rheotaxis as a response to the imprinted scent during hundreds of kilometres raises the question of how the fish avoid habituation to this scent. One possibility is that the fish regularly escape from the odor instead of being constantly exposed to it. Ascending adult salmonids near the region of confluence between two tributaries move along the interface between the two separate masses of water until they finally choose their native stream (Hasler and Scholz 1983). This horizontal zigzag is comparable to a vertical zigzag pattern of Atlantic salmon in coastal areas where the fish are also thought to follow a specific water layer containing home water (Døving 1989). However, waterfalls and rapids efficiently mix water masses, making such contrasts unavailable in long parts of the river. Another possibility could be that the fish do not habituate to this specific scent. Such a very low or nonexistent adaptation rate has been shown for the olfactory response of male catfish to the sex pheromone of females (Smith 1985). Finally, increasing olfactory sensitivity to the imprinted scent during the period of upstream migration, as shown by electroencephalogram response to home water or imprinted scent, could continuously counterbalance habituation (Hasler and Scholz 1983). The increasing concentration of home water as the fish progress, leaving other tributaries behind, probably also contributes to increasing olfactory input.

The scents to which fish are naturally attracted are still unknown. Nordeng (1971, 1977) proposed that anadromous salmonids possess an innate ability to recognize pheromones released by members of their own family and that maturing fish may be guided by population-specific pheromone trails released from descending smolts. Stock-specific substances from mucus and intestinal content have been proposed as olfactory tracers (Smith 1985). Compounds released from dead bodies of postspawners and being incorporated into the soil or plants could create a long-lasting, stock-specific river odor. However, the pheromone hypothesis has been challenged (Black and Dempson 1986; Hansen et al. 1993) and numerous experiments have proven that salmonids are able to home in the absence of pheromones due to a learning process (Hasler and Scholz 1983). This learning process may

include the recognition of organic and inorganic compounds which give each river its specific bouquet of odors (Brannon 1982). One may speculate that salmonids most probably recognize a set of odors, some genetically and others following imprinting, which allows for local adaptation and homing even when one of the odors is removed, either experimentally or accidentally. It would be of interest to include habituation level of olfaction to the tested substance in studies concerned with the research for appropriate substances involved in homing.

1.4.3. Visual references

Blinding has been reported to impair homing, although not as consistently as removal of olfaction (Døving 1989). There are several ways by which visual aerial references can be used as orientation cues by migrating fish (Smith 1985). Fish can see landmarks and celestial bodies through the water surface. When the water surface becomes disturbed, fish can detect the position of celestial bodies by observing the angles of beams cast through the surface by the sun and presumably the moon and the stars. Finally, when clouds cover the sun, fish can detect its position by sensing the pattern of polarization of light. They can either see the distribution of polarized light in the sky through the water surface or see the pattern of polarization of light under water. As light penetrates water, it is polarized in a distinctive pattern related to the sun's direction. This last information could be available at considerable depths (Smith 1985).

There is evidence that sockeye smolts use the sun as a visual reference, either directly or through the pattern of polarization of the sky (Smith 1985). Such orientation suggests that the fish possess a clock mechanism that enables them to correct for the sun's apparent movement during the day. There is evidence that landmarks are used in three different ways. Fish may recognize familiar regions, they may recognize general types of habitat, or they may maintain visual headings initially set on the basis of other information. The first type of information could be used by the fish in association with learning. In an experimental system, four out of nine Atlantic salmon parr learned to use a visual landmark to track a food resource (Braithwaite et al. 1996). The recognition of general types of habitat by fish has been exploited to direct migrants towards preferred visual configurations (Smith 1985). Finally, sockeye smolts have been shown to orient in a tank according to celestial cues and to remain oriented towards some marks on the tank after those celestial viewing conditions have changed, suggesting they use landmarks in the same way as a woodsman uses a compass (Smith 1985).

1.4.4. Magnetism

When celestial cues are absent, both sockeye smolts and fry have been shown to orient according to the magnetic field, indicating the presence of a magnetic compass with lower priority than the celestial compass (Smith 1985). The lower priority of the magnetic cue is probably related to its lower precision as compared to the sun, a feature which is apparent both in sockeye and in birds (pigeon) (Smith 1985). The nature of magnetic sensitivity in nonelectric fish has long been obscure. Recently, however, Walker et al. (1997) identified candidate magnetoreceptors in the nares of rainbow trout. The candidate receptor cells contain crystalline material which might be magnetite (Fe_3O_4). Chains of magnetite had earlier been extracted from the region of the ethmoid tissue in sockeye salmon (Mann et al. 1988). The receptor cells are connected to the brain through the trigeminal nerve and respond to changes in the intensity but not the direction of an imposed magnetic field (Walker et al.

1997). They are situated within the olfactory lamellae and "the apparent physical proximity of magnetoreception and olfaction raises the intriguing possibility that olfactory impairment would also produce magnetic impairment" (Walker et al. 1997). In particular, previous conclusions based on studies in which olfaction was suppressed by cauterizing the nares should be reconsidered on the basis of this new discovery.

In addition to the sensory system discovered by Walker et al. (1997), which was shown to react to changes in field intensity only, there could be magnetoreceptor cells at other localizations responding to directional changes of the magnetic field. Chains of magnetite in one single cell, allowed to align to the magnetic field and connected to a single sensory neuron, are sufficient to give the fish a good magnetic compass sense (Kirschvink 1997). Moore et al. (1990) identified magnetic material associated with the lateral line in Atlantic salmon and suggested that modified lateral line mecanoreceptors could serve as magnetoreceptors. The anterior 30% of the lateral line, posterior to the operculum, contained most magnetic material. Adults had more than smolts. The material was suggested to be single-domain particles of magnetite, of biogenic origin. No chains were found but the particles appeared larger than those previously found in fish. Electrophysiological studies remain to be done (Moore et al. 1990). Finally, there is some evidence that light-dependent magnetoperception may play some role in orientation of salmonids, since magnetic perception of retinal melanin was higher in migrating Pacific salmon than in nonmigrants (Zagal'skaya 1994).

1.4.5. Temperature

In mountain lakes, there may be predictable differences in temperature between the inlet and outlet rivers. Cooler waters from the inlet fill the deep portions of the lake whereas warmer surface waters flow on downstream through the outlet. Therefore, the temperature gradient through the lake could be used by salmonids during migration. However, the diffuse nature of the thermosensory system in fish precludes experiments based on its blocking or its destruction. The use of temperature gradients in migration is therefore still speculative (Smith 1985).

1.4.6. Conclusions

It is most probable that several mechanisms are involved in the homing mechanism, leading once more to a great plasticity and adaptability of salmonids. Species spending only a few weeks at sea and undertaking the same migratory route several times may not depend on such a strong imprinting as species undertaking a single migration and spending up to 3 or 4 years at sea. The brown trout, which may undertake repeated migrations, was the only species among seven cited in Smith (1985) that homed independently of whether olfaction was present or not. These brown trout may have depended on other cues. Arctic char may often spend the winter in different rivers but tend to return to their home river the year they spawn (T.G. Heggberget, NINA.NIKU, Trondheim, Norway, personal communication). Such behavior confirms the importance of sexual maturation on olfactory sensitivity and scent recognition (Hasler and Scholz 1983) and suggests that genetic factors may play a major role in determining the mechanisms involved in orientation. Repeated, independent, imprinting processes at each smoltification or downstream migration would be inappropriate in Arctic char.

The plasticity of homing extends to the development, within each population, of a certain degree of straying to other water courses, which allows for colonization of new areas,

ensures the survival of some members of a population following a catastrophe in the home river, and reduces inbreeding in small populations (Quinn 1993).

One important point is the finding that homing involves imprinted as well as genetic memory, and that downstream and upstream migration are both associated with a short and special period of brain and olfactory readiness, oriented towards scent learning or scent recognition, respectively. Whether this activation also includes other senses is not known. The role of thyroid hormones, cortisol, and sex steroids during these critical periods will be discussed in Chapter 4.

2. The transfer between river and sea

The highly adaptive character of salmonids, which has been illustrated in the previous chapter, is even more impressing when one considers the habitat change experienced by the fish as it moves between freshwater and seawater. Freshwater and seawater habitats differ in ionic composition (Schmidt-Nielsen 1990), oxygen and carbon dioxide solubility (Boutilier et al. 1984), and often in pH and buffering capacity (Heisler 1984). Whereas freshwater teleosts have body fluids many times more concentrated than their environment, seawater teleosts maintain the solute concentration of their body fluids distinctly below that of their environment. The mechanisms involved in fish osmoregulation have been reviewed by Evans (1979; 1993) and Wood and Shuttleworth (1995); therefore, only adaptive aspects linked to the transfer of salmonids between freshwater and seawater will be presented here. Salinity changes are also associated with adjustments in respiratory and circulatory variables, acid–base status, and metabolism, which have been far less studied than the osmoregulatory adjustments (Larsen and Jensen 1993; Soengas et al. 1995a).

Present knowledge on physiological adjustments following salinity changes is mainly based on direct transfer experiments of hatchery-reared fish between freshwater and seawater. In the wild, salmonid smolts and adults may either move rapidly between the two media or remain for some time in estuaries and thus experience a more gradual transfer (McCormick 1994; Moore et al. 1995; Greenstreet 1992c). Further, the existence of strong tidal currents may lead to complex water structures within some estuaries and the migrating fish may alternatively enter fresh and seawater for short periods of time (Chernitsky et al. 1993).

2.1. The transfer from freshwater to seawater

2.1.1. Osmoregulatory changes

When entering seawater, migratory salmonids are suddenly exposed to salt loading and water loss. The intensity and kinetics of the changes experienced are dependent upon external and internal variables, such as water temperature and ionic composition, fish size, species, and level of preadaptation. The present review was aimed at delineating the basal mechanisms involved in osmoregulatory adjustments to a hyperosmotic medium in euryhaline fish. If data were available, changes in preadapted smolts were compared to changes in nonpreadapted fish, most often rainbow trout. When no data on salmonids was found in the literature concerning adaptational changes believed to be important to this group of fish, results from other euryhaline species, such as the eel, *Anguilla* spp., or the tilapia, *Oreochromis* spp., are reported. Specific values obtained in single studies were reported in order to give an indication of the magnitude of changes that may occur. These values should, however, not be considered as general values since great variations exist.

2.1.1.1. Water and ion movements

Following abrupt transfer of euryhaline salmonids from freshwater to seawater, plasma salt concentrations typically increase during the first 24–48 h, after which they decrease to values similar to or slightly above freshwater levels (Houston 1959; Hegab and Hanke 1986; Finstad et al. 1988; Seddiki et al. 1995; Nonnotte and Bœuf 1995). Changes in plasma chloride, sodium and magnesium concentration are the most consistent and significant, whereas plasma potassium and calcium concentrations remain relatively stable (Stagg et al. 1989; Björnsson et al. 1989). The time-related variation in plasma chloride or sodium

following seawater transfer is routinely used as an index of seawater tolerance (Clarke 1982; Clarke and Blackburn 1978).

During the initial period, plasma chloride typically increases more than plasma sodium. In one study with nonpreadapted rainbow trout weighing 760–950 g, mean plasma chloride and sodium concentrations increased by 47 and 30%, respectively, 24 h after transfer to 35‰ seawater (Maxime et al. 1991). In another study with preadapted Atlantic salmon smolts weighing 52–58 g, mean plasma chloride and sodium concentrations increased by 9 and 8%, respectively, 48 h after transfer to 32‰ seawater (Nonnotte and Bœuf 1995). In these Atlantic salmon smolts, chloride and sodium concentrations returned to freshwater levels 2 weeks after transfer.

Osmotic loss of water is counteracted by a sharp increase in drinking rate and a drop in urine production resulting from decreased glomerular filtration rate. In rainbow trout abruptly transferred to seawater, urine flow was reduced to 25% by 1 h and stabilized at 1% of freshwater value after 4 h (Sinnott and Rankin 1976). In rainbow trout transferred to two-thirds seawater, the drinking rate increased from almost zero to 25 $mL \cdot kg^{-1} \cdot h^{-1}$ at 6 h after transfer, then rapidly declined towards the stable seawater level of about 5 $mL \cdot kg^{-1} \cdot h^{-1}$ at 8 h after transfer (Bath and Eddy 1979a).

The rate and magnitude of changes in body fluid electrolyte composition is restricted by an "osmoregulatory buffer system," which provides, at slight metabolic cost, the time interval necessary for the development of structures and functions adapted to osmoregulation in seawater (Houston 1964). This buffer system consists of a passive shift of fluid from intracellular to extracellular compartments, uptake of ions in soft tissues and bones, and a rapid decrease in surface permeability. In one study on the steelhead trout, the volume of the extracellular phase was found to expand by about 45%, while the volume of the intracellular phase was found to decrease by about 10%. These changes in the fluid compartments allowed the dilution of incoming extracellular electrolytes at the cost of a relatively small degree of cellular dehydration (Houston 1964). In one study on hatchery-reared coho smolts, blood haematocrit decreased by more than 20% at 24 h following seawater transfer (Shrimpton and Bernier 1994). Calcium level in muscle cells of steelhead trout rose sharply in seawater, although there was little change in the plasma concentration, suggesting increased uptake in tissues (Houston 1959). The rapid decrease in water permeability of surface epithelia has been suggested to result from increased calcium concentration and lowered pH (Houston 1964).

Progressively, upon transfer to seawater, newly synthesized or activated ion extrusion mechanisms efficiently decrease plasma concentrations of salts. During this active regulatory phase, intake of salts is reduced by a lower drinking rate. Redistribution of water and ions between body compartments occurs (Bath and Eddy 1979a). An adaptive rehydration of cells occurs, sometimes even a transient overhydration (Madsen and Naamansen 1989). In fully adapted steelhead trout, the intracellular phase volume was similar to that of freshwater-adapted fish, while the extracellular phase volume was about 10% higher (Houston 1964). Too few studies have been performed, however, to permit any generalized hypothesis on fluid movements following salinity changes in salmonids (Olson 1992). The impact of such fluid shifts on the concentration of plasma components, including hormones, are therefore difficult to assess.

In smolts, preadaptation includes a change in the functional characteristics of the gills towards ion extrusion. In a perfused-isolated head of Atlantic salmon smolt, sodium net flux from blood to water was 68 $mmol \cdot h^{-1} \cdot 100g^{-1}$ in freshwater and increased to 1265 $mmol \cdot h^{-1} \cdot 100g^{-1}$ after contact with seawater, in spite of a passive influx of

2105 $mmol \cdot h^{-1} \cdot 100g^{-1}$ in seawater (Avella and Bornancin 1990). Thus, smolts loose small amounts of sodium through the gills in freshwater and increase sodium outflux by a factor of 45 once being exposed to seawater. The mechanism of this activation is still unknown but is thought to relate to the NaCl concentration rather than the calcium concentration or the osmolarity of the medium (see Avella and Bornancin 1990). The net loss of sodium in freshwater-adapted smolts may be due to increased ion permeability of the gills. It may be associated with an increase in whole body water content and a strong dependency on dietary intake of salts since starvation in Atlantic salmon smolts may result in a large decline in plasma osmolality (Duston et al. 1991).

In rainbow trout, a protective effect of starvation on seawater transfer has been demonstrated (Nance et al. 1987). Whereas a survival rate of 12% after direct transfer into seawater was observed in fed fish, fasting for 3 and 10 days increased survival rate to 50 and 100%, respectively. Starvation was associated with changes in transbranchial fluxes and in the dynamics of structural changes of gill epithelium (Nance et al. 1987).

2.1.1.2. Structural and morphological changes

A number of structural and morphological changes have been described in the osmoregulatory organs of euryhaline fish during preadaptation and adaptation to seawater. It is important to keep in mind, however, that many functions are regulated and the presence of a structure does not mean it is fully activated. This is particularly important when interpreting enzyme activity data. The well-known Na^+K^+-ATPase activity, for example, is in most cases measured *in vitro* from homogenized tissues under standardized conditions. Values observed represent the functional capacity under optimized *in vitro* conditions and are related to existing structures, particularly to the number of sodium–potassium exchanger pumps (McCormick 1995). They represent by no means the true *in vivo* activity, which may be regulated by a number of factors. In addition to the local concentrations in sodium, potassium, and ATP, which have obvious regulatory effects, there is some evidence that stores of phosphocreatine and plasma cortisol concentration could be implicated in short-term regulation of Na^+K^+-ATPase in fish (McCormick 1995; Kültz and Somero 1995). For recent reviews on the short-term regulation of Na^+K^+-ATPase in mammals, see e.g., reviews by Bertorello and Katz (1993) and Ewart and Klip (1995). A number of studies do indeed indicate that gill Na^+K^+-ATPase activity, as measured *in vitro*, is not always a good indicator of salinity tolerance or branchial NaCl excretory ability (for references, see Duston et al. 1991).

Gills

In freshwater, the gills have a low permeability to water and ions, while in seawater, the gills may be less permeable to water but are much more permeable to ions (Evans 1993). The greatest structural changes occurring in the gills concern the chloride cells. These cells cover less than 10% of the surface but are responsible for most monovalent ion exchanges both in freshwater and seawater (for details on the structure and function of the gills, see reviews by Laurent and Dunel 1980, Foskett et al. 1983, and Perry and Laurent 1993). During seawater adaptation, chloride cells show both hyperplasia and hypertrophy, the latter being associated with increased differentiation of mitochondria and increased synthesis and incorporation of plasma membrane into the baso-lateral tubular system, paralleled by increases in Na^+K^+-ATPase activity. Studies on the tilapia opercular membrane, which is largely used as an experimental model for teleost gills, suggest the existence of two phases: proliferation, followed by differentiation (Foskett et al. 1983). During the first three days, existing chloride cells are activated and proliferate. Then, the cells stop proliferating and

enlarge for at least three weeks, concomitantly with the development of the baso-lateral membrane tubular system and of mitochondria. The chloride current through these cells is activated within 24 h following transfer and increases steadily during the two phases. Fully adapted levels are reached after 1–2 weeks (Foskett et al. 1983).

The apical surface of chloride cells changes following seawater transfer. According to Perry and Laurent (1993), this epithelial structure shows the most obvious and consistently observed difference between freshwater and seawater-adapted fish. In freshwater, the apical membrane of chloride cells is either in alignment with, or slightly above, that of the adjacent pavement cells; in contrast, in seawater it forms an apical crypt so the area exposed to the external medium is reduced to a narrow apical pit. The functional advantage of the configuration seen in seawater may be to impede passive inward diffusion of electrolytes by restricting water convection in this area (Perry and Laurent 1993). The apical membrane receives interdigitations originating from the baso-lateral membrane of seawater-specific neighboring cells, the "accessory" or "companion" cells (Perry and Laurent 1993). Chloride cell and accessory cell membranes are joined by a single-strand "leaky" junction, highly permeable to electrolytes. The development of these leaky junctions is apparently a prerequisite for successful transfer of euryhaline species from freshwater to seawater and is believed to play a major role in the paracellular efflux of sodium (Perry and Laurent 1993).

Isaia (1984) proposed that increased ionic movements could cancel the osmotic gradient across the gills, thereby preventing dehydration in marine fish. In concordance with this view, the osmotic movement of water, when measured as the equivalent of the oral ingestion rate in seawater and as the urine flow in freshwater, seems to be of the same order in the two salinity extremes, despite the smaller osmotic gradient in freshwater (Evans 1979). Following transfer to seawater, the gill epithelium is transformed "from a relatively impermeable, nontransporting tissue in freshwater to one in seawater dominated by cells with some of the highest ionic permeabilities and transport rates ever recorded" (mean surface current of 18 $mA \cdot cm^{-2}$ and mean conductance of 580 $mS \cdot cm^{-2}$) (Foskett et al. 1983). Chloride efflux in Atlantic salmon smolts took about 18 h to reach seawater levels (Potts et al. 1970). In rainbow trout, the time needed for unidirectional sodium fluxes to reach seawater levels was of the same order of magnitude (Hegab and Hanke 1986). In contrast, gill Na^+K^+-ATPase activity in the same species increased during 6 days after transfer (Madsen and Naamansen 1989). The chloride cells possess a well-developed vesicular system in the apical region of the cells (Laurent and Dunel 1980). On the basis of the observed kinetics, it is tempting to suggest that the development of interdigitations and thus leaky junctions is based upon a rapid organization of preexisting vesicular membrane, whereas, the amplification of baso-lateral membrane associated with the increased Na^+K^+-ATPase activity is based upon newly synthesized membrane material, a process that is more time demanding (Kaissling and Kriz 1985).

Finally, the rate of renewal of both pavement and chloride cells is markedly stimulated in seawater-adapted fish. In fully acclimated fish, the increased rate of cell turnover is the result of accelerated differentiation and apoptosis. In fish acutely transferred from freshwater to seawater, there is an additional component of cellular necrosis (Perry and Laurent 1993). In rainbow trout, a "flattening wave" that propagates along both primary and secondary lamellae has been described (Nance et al. 1987). It is thought to be a degenerative process, which could be associated with the replacement of freshwater-adapted chloride cells with seawater-adapted ones (Nance et al. 1987).

In rainbow trout, gill Na^+K^+-ATPase activity was increased 4 days after transfer to a salinity of 28‰ but not 20‰ (Fuentes et al. 1997). Thus a rapid structural increase in

Na^+K^+-ATPase occurred only above a salinity threshold much higher than isotonicity (Fuentes et al. 1997). Because plasma sodium concentration was well regulated in both cases, the required increase in ion extrusion at 20‰ was probably associated with an activation of existing pumps. In contrast, in long-term adapted Atlantic salmon smolts, gill Na^+K^+-ATPase activity was higher at isotonicity (10‰) than at 0‰ (McCormick et al. 1989a). The physiological function of such an increase is unknown but suggests that long-term adaptation through increased capacity of the enzyme is positively correlated to salinity, perhaps via corticosteroid-induced synthesis of the enzyme (McCormick et al. 1989a). The base level of gill Na^+K^+-ATPase and the rapidity of the increase following seawater transfer are higher in smolts than in parr or post-smolts (Madsen and Naamansen 1989). These observations suggest the existence of a complex regulation of structural adaptations to seawater.

Esophagus

Seawater adapted fish compensate for osmotic losses by drinking seawater. Seawater is desalinated in the esophagus by passive diffusion and active transport of sodium and chloride, while water is retained in the lumen (Evans 1979; Kirsch and Meister 1982; Parmelee and Renfro 1983). Changes in the structure of the esophagus have been studied during seawater adaptation of the Japanese eel, *Anguilla japonica* (Yamamoto and Hirano 1978). The freshwater-adapted eel esophagus has a stratified epithelium, rich in mucous cells, similar to the epithelium of the oral cavity and epidermis, and impermeable to both water and ions (Evans 1979). Following transfer to seawater, the esophageal epithelium is replaced by a simple columnar epithelium free from mucous cells, permeable for ions but impermeable for water. Its surface area is increased by enhanced folding and a high vascularization of the connective tissue layer develops beneath the columnar cells. Similar hyperemia has been observed in seawater adapted flounder, *Pseudopleuronectes americanus*, as compared to flounder adapted to 10% seawater (Parmelee and Renfro 1983). Three or four days after transfer of eels from freshwater to seawater, large amounts of cellular debris from the mucosal surface, blood cells, and mucus were observed in the esophageal lumen (Yamamoto and Hirano 1978). One week after transfer, simple columnar epithelium was present in several places, while two weeks after transfer, most of the mucosal surface was composed of columnar epithelium. The columnar cells of seawater eel were separated by prominent intercellular spaces sealed on the mucosal side by junctional complexes. These probably reflect the active extrusion of ions through the baso-lateral membrane of the esophageal cells, causing osmosis towards the interstitial spaces (Yamamoto and Hirano 1978).

Intestine

Water uptake occurs through the small intestine, following active uptake of sodium and chloride via a $Na^+K^+2Cl^-$ cotransport system (Evans 1993). This intestinal salt and water transport has been shown to increase largely as rainbow trout are exposed to increasing salinity (Shehadeh and Gordon 1969). In seawater-adapted coho salmon, 95% or more of the water absorption occurred in the anterior intestine and pyloric caecae (Kerstetter and White 1994). The mid and posterior intestine may, however, be important in water absorption during the initial stages of seawater adaptation. *In vitro* fluxes through these sections changed following seawater transfer of coho smolts and complete adaptation of intestinal transport mechanisms required several weeks (Kerstetter and White 1994).

Morphological changes in the middle intestine of rainbow trout following seawater transfer have been described by Nonnotte et al. (1986). Important modifications occurred shortly after transfer. After two days, significant distension of the intercellular spaces could be observed concomitant with an increase in intestinal absorption of sodium and chloride

and suggestive of an increase in paracellular water and ion flow from lumen to blood. In addition, numerous tubular invaginations of the baso-lateral membrane appeared, similar to those seen in specialized salt-transporting cells (Kaissling and Kriz 1985). At the same time, a two-fold increase of Na^+K^+-ATPase activity per unit area of serosal surface occurred (Nonnotte et al. 1984). The specific activity of the Na^+K^+-ATPase, relative to total protein content of the membrane, was unchanged two days following transfer but increased after one week (Nonnotte et al. 1987). After adaptation for one month in seawater, both the distention of the intercellular spaces and the tubular invaginations had disappeared. The middle intestine had recovered a structure very similar to that of freshwater adapted fish, except that the number of mucous cells had decreased. Nonnotte et al. (1986) suggested that long-term intestinal adaptation to an hypertonic environment could be based mainly on renewal of membrane components rather than on the development of new cellular structures. In concordance with this view, the proportion of (n-3) PUFA in the gut was significantly higher one month after seawater transfer of masu salmon, *O. masou*, smolts, as compared to freshwater smolts and control fish kept in freshwater (Li and Yamada 1992).

Smoltifying species may anticipate necessary intestinal changes prior to seawater transfer. In one study on Atlantic salmon, Usher et al. (1991a) observed a two-fold increase in mucosal to serosal water transport across the middle intestine during smoltification and no further increase over a period of 20 days in seawater. This transport was ouabain sensitive, indicating an increase in gut Na^+K^+-ATPase activity during smoltification, just as found in the gills. In contrast, Veillette et al. (1993) found a decrease in fluid uptake through isolated midgut in Atlantic salmon smolts, as compared to parr, and proposed that the changes reported by Usher et al. (1991a) may have been the result of seasonal changes. In isolated posterior intestine, however, Veillette et al. (1993, 1995) also found an increase in fluid uptake during smoltification as well as following seawater adaptation of smolts. These authors suggested that smoltifying salmon may experience a regionalization of the intestine, "with perhaps the middle intestine participating more in nutrient uptake and less in salt and water balance." This regionalization of the mid and posterior parts of the intestine could play some role in osmoregulation at least during the initial period of seawater adaptation.

As in the gills, intestinal gill Na^+K^+-ATPase activity *in vitro* was increased 4 days after transfer to a salinity of 28‰ but not to 20‰ (Fuentes et al. 1997). Baso-lateral Na^+K^+-ATPase activity generates the electrochemical gradient for Na^+ which drives the $Na^+K^+2Cl^-$ co-transport (Evans 1993). Here again, an activation of existing enzymes could be sufficient at low salinity to ensure sufficient water intake.

Kidney

The main function of the teleost kidney is the excretion of large amounts of diluted urine in freshwater and the excretion of divalent ions with a minimal water loss in seawater (Evans 1979). In the eel, clear histological changes occurred in the kidney during the first two days following seawater transfer, while a stable structure was reached around the 20th day (Olivereau and Olivereau 1977). These changes included a slight decrease in glomerular size with an increased amount of mesengial tissue and a marked reduction of the epithelial height along the nephron. The brush borders became thinner and basal folds and mitochondria volume were reduced. Phospholipids were less abundant. Changes were greatest in distal segments and collecting tubules, which are involved in dilution of urine in freshwater (Evans 1979).

The morphological changes observed in the kidney may, however, play only a minor role in seawater adaptation, since the two species of tilapia, *Oreochromis mossambicus* and

O. niloticus, underwent similar changes in glomerular and tubular structure following seawater transfer despite their significantly different seawater tolerance (Cataldi et al. 1991). In contrast, changes in the structure of the esophagus in the two species showed differences that were more directly related to their salinity tolerance (Cataldi et al. 1991).

2.1.2. Acid–base status, respiratory, and circulatory variables

Changes in acid–base status, respiratory, and circulatory variables following seawater transfer have been described in several salmonid species. Many of these changes may be assigned to osmoregulatory perturbations and ionic readjustments. Acid–base regulation is coupled to ionic exchanges across the gills, thus ionic readjustments may affect acid–base status. According to Nonnotte and Bœuf (1995), however, such exchanges can not, by themselves, explain the acid–base disturbances observed; the mechanisms involved in these salinity-dependent acid–base disturbances are complex and remain unknown. One important phenomenon may be shrinkage of the gills, which may alter their function as respiratory organs and acid–base regulators and increase branchial vascular resistance. Stagg et al. (1989) suggested that one major aspect of smolting was the ability to rapidly regulate branchial dehydration, normalizing ion exchange mechanisms and gas transfers. Indeed, Atlantic salmon smolts show only slight and short changes in plasma oxygen content and acid–base status following seawater transfer, as compared to parr or to nonsmoltifying rainbow trout (Stagg et al. 1989; Maxime et al. 1991; Seddiki et al. 1995). In Atlantic salmon, anoxia and hypertension following seawater transfer is high in parr and absent in smolts (Smith et al. 1991). The effects of changes in branchial integrity on the capacity to transport respiratory gases and to excrete acid or base equivalents by the gill are, however, largely unknown (Stagg et al. 1989) and are obviously an area for further work.

2.1.2.1. Acid–base status

The freshwater to seawater transfer of large cannulated rainbow trout was associated with an acidification of plasma, plasma pH decreasing from 7.94 to 7.67 at 24 h after transfer in one study (Maxime et al. 1991) and from 7.93 to 7.61 at 4 days after transfer in another (Seddiki et al. 1995). During the first hours after transfer, however, a transient alkalization of plasma was reported in rainbow trout and Atlantic salmon smolts, possibly due to putative alterations in ion exchange mechanisms through the gills due to an intense shrinkage of gill tissue (Stagg et al. 1989; Larsen and Jensen 1993). In Atlantic salmon smolts, pH rapidly returned to freshwater levels (Stagg et al. 1989), whereas in rainbow trout, pH decreased towards an acidosis (Maxime et al. 1991; Seddiki et al. 1995). In Atlantic salmon parr, which were unable to osmoregulate and possibly to control branchial dehydration, the alkalosis persisted until the end of the experiment (48 h) (Stagg et al. 1989).

The acidification of plasma could in some cases be totally explained by the higher influx of chloride than sodium (Maxime et al. 1991). This causes a net gain of negative charges, which is compensated for by a decrease in plasma bicarbonate and carbonate ion concentration, inducing a decrease in plasma pH (Maxime et al. 1991). The magnitude of the change in plasma pH is therefore probably linked to the osmoregulatory ability of the fish at the time of the transfer and to the degree of salinity. Indeed, transfer of rainbow trout to two-thirds seawater depressed pH by only 0.12 pH units compared to freshwater values (Larsen and Jensen 1993). The acidification of plasma may sometimes be enhanced by an increase in plasma lactate (Seddiki et al. 1995) or in P_{CO_2} (Larsen and Jensen 1993), which

are thought to result from reduced ventilatory capacity of the gills following seawater transfer (Stagg et al. 1989).

Recently, Perry and Laurent (1993) proposed that during acidosis, vesicles coated with putative proton pumps from the Golgi of gill pavement cells fuse with the apical membrane, enhancing outward proton transport. Such adaptation may occur much more rapidly than phenomena involving newly synthesized membrane (Kaissling and Kriz 1985). During respiratory acidosis in rainbow trout, the exposed surface of pavement cells expand at the expense of the exposed surface of chloride cells, allowing for a high density of proton pumps (Perry and Laurent 1993). The position of chloride cells in seawater, being recessed within the filament epithelium (see earlier), would favor such a mechanism. Whether this mechanism is functional during seawater adaptation is, however, unknown.

2.1.2.2. Respiratory variables

Several changes in respiratory variables may occur following seawater transfer. Salinity, as well as carbonation and temperature, affect the solubility of oxygen and carbon dioxide in water (Dejours 1981). At similar temperatures and within the range of pressure prevailing at the level of the gill, seawater usually has a lower content of oxygen than freshwater (Maxime et al. 1990). In addition, chloride cell proliferation in gills during seawater adaptation may benefit ionic regulation at the expense of efficient gas transfer. Induction of chloride cell proliferation by cortisol and growth hormone treatment in freshwater rainbow trout induced elevated P_{CO_2} values, a significant acidosis, and higher amplitude of ventilation movements (Bindon et al. 1994a). The expansion of pavement cells above the chloride cells, as described earlier (see section 2.1.1.2), may be a means to limit the negative impact of chloride cell proliferation on gas exchanges. Homeostatic disturbances may also affect respiratory variables. Immediate shrinkage of the gills following seawater transfer in one study, caused a 33% decrease of respiratory area as well as a possible compression of branchial vessels (Bath and Eddy 1979b). In addition, both increased plasma chloride concentration and acidosis decrease the affinity of haemoglobin for oxygen (Jensen et al. 1993).

Transfer to seawater of freshwater adapted rainbow trout was immediately followed by a transient decrease in arterial P_{CO_2} (Bath and Eddy 1979b; Maxime et al. 1991). To compensate for this decrease, ventilatory flow increased 55% by 1 h (Maxime et al. 1991). P_{O_2} in arterial blood returned to freshwater levels by 6 h. However, the arterial oxygen content gradually decreased by 38% after 24 h, due to a marked decrease in the affinity of haemoglobin for oxygen. This explained the maintenance of an elevated respiratory flow, which was still 32% above freshwater level 24 h after transfer (Maxime et al. 1991).

The transient decrease in arterial P_{O_2}, which may last for 24 h in some cases, is paralleled by a decrease in the nucleoside triphosphate concentration of red blood cells, a change which tends to increase blood oxygen affinity (Larsen and Jensen 1993). The rapidity of the nucleoside triphosphate response suggests that catecholamines or some unidentified homeostatic parameters are involved in the regulation of red cell nucleoside triphosphate content (Larsen and Jensen 1993).

2.1.2.3. Cardiovascular adjustments

In one study on rainbow trout, branchial vascular resistance initially increased by 28% and cardiac output slightly decreased following transfer to seawater (Maxime et al. 1991). These changes were, however, reversed at 6 and 24 h after transfer. At 24 h, branchial vascular resistance was 18% lower than in freshwater controls, systemic vascular resistance was 42% lower, cardiac stroke volume was 28% higher, cardiac output 30% higher, and

heart rate was almost unchanged. The decrease in systemic vascular resistance allowed for an increase in cardiac output, favoring oxygen delivery to tissues (Maxime et al. 1991).

2.1.3. Metabolic changes

During seawater adaptation, most salmonids show a decrease in food intake (Usher et al. 1991b; Arnesen et al. 1993; Soengas et al. 1995b; Fuentes et al. 1997). Concomitantly, the metabolic demands are high due to energy-consuming ion transport mechanisms and the restructuration of tissues towards seawater adaptation.

2.1.3.1. Oxygen consumption

Total oxygen consumption was found to be lower in the first hours following seawater transfer of rainbow trout (Bath and Eddy 1979b). Twenty-four hours after transfer, however, it had returned to freshwater level (Bath and Eddy 1979b). In the same species, other authors found an increase in oxygen consumption 24 h following seawater transfer (Maxime et al. 1991; Seddiki et al. 1995). In one of these studies, a 50% increase in standard oxygen consumption was observed at 6 h after transfer, followed by its return to freshwater levels at 4 days after transfer (Seddiki et al. 1995). The changes in oxygen consumption could be associated with a transient increase in energetic cost of ventilation and osmoregulation during the initial phase of seawater transfer. In isolated gill filaments, oxygen consumption increased by about 20% when incubated at 30‰ as compared to 0‰, independent of the prior acclimation salinity (McCormick et al. 1989a). No long-term changes in metabolic capacity of the gills was found in that study (McCormick et al. 1989a). These observations suggest that mainly the initial phase of seawater transfer is associated with increased energetic demand in gills.

2.1.3.2. Carbohydrates

Transient increases in plasma concentration of glucose, and less commonly lactate, have been reported following seawater transfer (Madsen 1990a; Soengas et al. 1995a; Seddiki et al. 1995). Soengas et al. (1995a, 1995b, 1995c) studied carbohydrate metabolism in liver, gill, and muscle of small and large rainbow trout that were gradually transferred to seawater. Glycogenolysis increased in the three organs studied. Gluconeogenesis increased in the liver of large fish only. Plasma glucose concentration increased with increasing salinity, but the utilization of exogenous glucose increased only in the gills. Glycolysis increased both in the gills and the muscles. Since the swimming activities were similar in seawater-transferred fish and freshwater controls, the authors proposed that the degradation of muscle glucose was directed towards exportation of lactate to osmoregulatory organs, such as the gills. Lactate functions as an excellent fuel for gill cells (Mommsen 1984; Soengas et al. 1995a) and may represent an important supplement to blood glucose and to the small local carbohydrate stores to fuel the energy-demanding processes associated with ion regulation. Whereas large fish mobilized glucose only from white muscle, small fish, probably less fit for seawater transfer and possessing a smaller relative amount of muscle (Videler 1993), also showed high glycolysis in red muscle (Soengas et al. 1995b).

Mobilization of liver glycogen has been observed in other salmonids, such as coho and Atlantic salmon (Plisetskaya et al. 1991). In contrast to coho salmon, liver glycogen following seawater transfer of Atlantic salmon remained low for at least three months (Plisetskaya et al. 1991), suggesting the existence of species differences in carbohydrate metabolism following seawater transfer.

The pentose phosphate shunt was activated in the gills, unchanged in muscle, and temporarily depressed in the liver (Soengas et al. 1995a, 1995b, 1995c). Because the pentose phosphate pathway generates increased reducing power necessary for membrane lipid synthesis, its activation in the gills could be related to the changes in gill structure occurring after seawater transfer (see section 2.1.1.2).

2.1.3.3. Lipids

Seawater adaptation of juvenile salmon is associated with a mobilization of triacylglycerols (Sheridan 1988a; Sheridan 1989; Li and Yamada 1992). A depletion of fat stores in liver, muscle, and mesenteric fat occurs normally during smoltification but is enhanced in early smoltification by seawater transfer (Sheridan 1988a). In fully smoltified fish, the depletion of these organs may have reached a maximal level, since no further depletion of fat from liver, muscle, and mesenteric fat occurred after seawater transfer of coho and chinook salmon (Sheridan 1988a). Following seawater transfer of masu salmon smolts, no changes in lipid content of liver and muscle occurred but triacylglycerol content in gills and gut decreased (Li and Yamada 1992). Thus local stores in vital osmoregulatory organs such as gills and gut were not depleted prior to seawater transfer. The depletion of fat stores occurring during smoltification is due both to an activation of triacylglycerol lipase activity and to a decrease in triacylglycerol synthesis (Sheridan 1989). The exact biological processes supported by the mobilized energy are unknown, although some role in fueling hypoosmoregulatory adjustments is probable. Lipase activity remained elevated in seawater smolts (Sheridan 1988a) and lipid content in muscle, liver, gut, and gill was lower in seawater smolts compared to control fish that had remained in freshwater (Li and Yamada 1992).

The fatty-acid composition of lipids changes during smoltification and following seawater transfer (Bergström 1989; Ogata and Murai 1989; Sheridan 1989; Li and Yamada 1992; Bell et al. 1997). Whereas parr are characterized by relatively high proportions of saturated fatty acids and low proportions of polyunsaturated fatty acids (PUFA), smolts are generally characterized by relatively low proportions of saturated fatty acids and high proportions of long-chain PUFA (see Sheridan 1989). Following seawater transfer of masu smolts, a significant increase in the proportion of (n-3) PUFA occurred in the gut, but not in muscle, liver, and gills (Li and Yamada 1992). However, Bell et al. (1997) found that the level of arachidonic acid (20:4n-6), eicosapentaenoic acid (20:5n-3), and docosahexaenoic acid (22:6n-3) in liver phospholipids increased following seawater transfer of Atlantic salmon.

In isolated salmon cells, the fatty-acid composition of glycerophospholipids changed when the cells were exposed to increasing salinity within the physiological range for plasma ionic content (Tocher et al. 1995). Thus environmental salinity seems to have a direct effect on cell membrane composition. The physiological consequences of such changes for salmonids have not been established. Possible effects include an alteration in osmotic resistance of the cells, an effect on cell volume regulation, and important effects on membrane-bound enzymes such as Na^+K^+-ATPase (Tocher et al. 1995). Smolts retained in freshwater return to a freshwater lipid pattern, which supports the importance of lipid composition for osmoregulation (Li and Yamada 1992). The dietary lipid content affects prostaglandin synthesis in gill cells and plasma chloride concentrations following seawater challenge (Bell et al. 1997). Leray et al. (1984) showed that increased polyunsaturated/saturated ratio in the gut brush border of rainbow trout following seawater transfer was concomitant with a significant increase in membrane fluidity. These changes occurred in the absence of any

variation in cholesterol content or phospholipid polar headgroups, pointing to the importance of fatty acids metabolism for seawater adaptation (Leray et al. 1984).

2.1.3.4. Growth

Under natural conditions, the seaward migration of salmonids is often associated with a sudden increase in growth rate. Atlantic salmon smolts, which may take 2–7 years to reach a size of 25–50 g, frequently attain weights of 1.5–2.5 kg during their first year at sea and may reach a weight of 20 kg during a 3-year period at sea (Borgstrøm and Hansen 1987; McCormick and Saunders 1987). Anadromous Arctic char from Halselva showed a 50–100% increase in body weight during 5–6 weeks of seawater residence and a 12–20% loss in weight during the 10–11 months of freshwater residence (Finstad and Heggberget 1993).

In hatchery-reared salmon, the seawater transfer is commonly associated with a temporary decrease in food consumption and growth, which may be followed by an increased growth rate. Stead et al. (1996) showed that Atlantic salmon fed a high ration level doubled their food intake and growth rate after being transferred to seawater.

Several observations, however, suggest that the transition of fish into a hyperosmotic medium does not favor growth *per se*. In a long term study, Austreng et al. (1987) showed that the growth rate of hatchery-reared Atlantic salmon and rainbow trout at a given temperature decreased steadily during their life span, thus being higher during the freshwater phase than during the seawater phase. The growth of hatchery-reared Atlantic salmon smolts and postsmolts retained in freshwater may be as great or greater than in those reared at various salinities up to 31‰ (Blake et al. 1984; McCormick et al. 1989b; Duston 1994). Similarly, the growth rate of Arctic char in April was independent of water salinity (0, 10, 15, 20, 25, 30, and 35‰) (Arnesen et al. 1993). In the wild, there are many examples of resident fish being larger than anadromous ones (McDowall 1988). For example, in Norway, anadromous Arctic char never exceed 4–5 kg, whereas resident populations may reach 10–12 kg (see Borgstrøm and Hansen 1987).

High growth at sea could be related to increased food availability or quality (McCormick et al. 1989b), more favorable temperatures (Austreng et al. 1987; Stead et al. 1996), and possibly higher swimming activity, which reduces agonistic behavior and increases protein synthesis and deposition in all tissues (Jobling 1994). In smoltifying species, the completion of smolting may be associated with an inflection of growth rate (Duston 1994), which under natural conditions corresponds to seawater entry.

In the wild, another major aspect of growth at sea is "catch-up growth," also referred to as "compensatory" or "recovery" growth (see Jobling 1994). Following a period of fasting, spawning, or other causes of energy depletion, fish and other animals are able to show marked growth spurts as food supplies increase. This appears to relate both to increased appetite and increased food-conversion efficiency. The importance of this phenomenon on seawater growth is illustrated by the study of Finstad and Heggberget (1993) who showed that well-fed, hatchery-reared Arctic char showed almost no growth at sea the year of release, as compared to wild fish. The following years, however, these fish had a condition factor as low in spring as the wild fish and their growth at sea was similar to that of the wild fish.

2.2. The transfer from seawater to freshwater

Compared with the large number of publications concerning the seawater transfer of salmonids, few experimental data have been published concerning their freshwater transfer.

2.2.1. Osmoregulatory adaptations

2.2.1.1. Water and ion movements

In one study, plasma ion concentration decreased rapidly following abrupt freshwater transfer of migrating adult Atlantic salmon caught in coastal seawater (Talbot et al. 1989). Minimal levels were reached within 24 h and more than 8 days were required for these variables to reach stable freshwater levels. Plasma osmolality fell by 35%, from 408 $mOsm \cdot kg^{-1}$ to 267 $mOsm \cdot kg^{-1}$, 60 h following abrupt freshwater transfer, then increased to 285 $mOsm \cdot kg^{-1}$ by 8 days and to 311 $mOsm \cdot kg^{-1}$ after 3 months. Plasma level of sodium declined by 39% 25 h after transfer. After 8 days, plasma levels of sodium and chloride were 33 and 34% lower, respectively, than in seawater; after 3 months, they were 25 and 21% lower, respectively, than in seawater (Talbot et al. 1989).

Total sodium efflux in seawater-adapted adult Atlantic salmon has been estimated to be 3.8 $mmol \cdot kg^{-1} \cdot hr^{-1}$ or 12% of total sodium content per hour (Potts et al. 1989). Following freshwater transfer, sodium efflux remained at that level during the first minutes, then rapidly decreased during the first hour, reaching an equilibrium level of 0.07 $mmol \cdot kg^{-1} \cdot hr^{-1}$ by 24 h after transfer, or 0.3% of total sodium content per hour. The mechanisms involved in this decline are thought to be a rapid reversal of the diffusion potential, the inner side of the gill epithelium becoming negative compared to its outer side (Evans 1984), a shutting down of the chloride pump, and finally changes in the structure of the tight junctions between chloride cells and accessory cells (Potts et al. 1989). Sodium uptake increased immediately to the freshwater level (0.143 $mmol \cdot kg^{-1} \cdot hr^{-1}$) in adult Atlantic salmon transferred to freshwater (Potts et al. 1989). The cumulative net loss of sodium was 18% of total body sodium during the first 6 h. During this period, plasma sodium declined by only 6%, suggesting that a shift of water occurred from the extracellular to the intracellular compartment, acting as a buffer to limit the decrease in plasma ion concentration. Homeostatic "buffering" opposite to that described by Houston (1964) for seawater transfer (see 2.1.1.1. Water and ion movements) seems to be of importance during freshwater transfer as well.

Just as the gut is the main osmotic effector in marine teleosts, the renal complex is the main osmotic effector in freshwater teleosts (Evans 1984). Renal adjustment of urine production seems to require a longer time than adjustment of ion transfer through the gills. When adult salmon were transferred from seawater to freshwater, an immediate influx of water occurred, while urine production remained at seawater level, 0.7 $mL \cdot kg^{-1} \cdot hr^{-1}$, for a day or more (Talbot et al. 1989). This caused a weight gain of 6% after 8 h and 12% after 24 h in adult Atlantic salmon (Potts et al. 1989). This period of water loading was followed by a six-fold increase of urine flow rate at 60 h after transfer of adults, then by a decline in urine production to the stable freshwater level, 1.2 $mL \cdot kg^{-1} \cdot hr^{-1}$, at 8 days after transfer. These changes were paralleled by similar changes in glomerular filtration rate, which remained about twice as high as urine flow (Talbot and Potts 1989).

Prior to the increase in urine production, the secretory and excretory functions of the nephrons changed (Talbot and Potts 1989). Within 25 h following transfer, the urine chloride and magnesium concentrations sharply declined, whereas urine sodium concentration remained constant. The ratio between urine and plasma concentrations of chloride was 0.7 in seawater-adapted fish and decreased to a freshwater level of 0.1 by 60 h. The ratio between urine and plasma concentrations of sodium decreased from 0.15 in seawater to 0.08 in long-term freshwater-adapted fish. Urine osmolality was 82% of that of plasma in seawater, 51% after 60 h in freshwater, and 14% of plasma osmolality in long-term adapted freshwater fish (Talbot and Potts 1989).

Talbot et al. (1989) suggested that the slow renal response to freshwater transfer may be of importance when salmon migrate through inshore waters, where they may experience transient salinity changes. One may further speculate that a delay in increasing urine production is important to allow the development, during the latency period, of structures necessary for active reabsorption of ions through the nephrons and urinary bladder. If urine production increased immediately following freshwater entry, cumulative salt losses through urine would probably be higher.

An interesting case is the conservation of magnesium during the first week(s) of freshwater transfer in adults, as shown in one study with Atlantic salmon. After transfer to freshwater, the plasma concentration of magnesium remained at seawater level, 5–6 $mmol \cdot l^{-1}$, for at least 8 days, while decreasing to 0.8 $mmol \cdot l^{-1}$ after long-term adaptation (Talbot et al. 1989). Magnesium excretion by the nephron declined within 24 h, allowing for high magnesium to be maintained in freshwater. The ratio between urine and plasma concentrations of magnesium was 28 in seawater, 1 by 60 h after transfer, and reached freshwater level of 0.5 after more than 8 days. In contrast, in Atlantic salmon smolts, immediate changes in plasma magnesium occurred following transfer to seawater and back to freshwater, seawater and freshwater levels being almost reached by 60–90 min (Chernitsky et al. 1993). Less than 15% of ingested magnesium is normally absorbed through the gut and all of the absorbed magnesium is excreted renally (Evans 1993). These results, therefore, suggest that the capacity of the kidney to adapt to salinity change is different in smolts and adults and that magnesium excretion is regulated by some other factor than plasma magnesium concentration, at least in adults.

2.2.1.2. Structural changes

Whereas entry of freshwater fish into seawater is limited by the presence or absence of an ionic extrusion system, the major limitation to entry of marine species into reduced salinities is the balance between salt loss and uptake, because the uptake system is active in seawater to regulate acid–base status (Evans 1984). At least in eel and mullet, *Mugil capito*, this change in balance is associated with a dedifferentiation of the chloride cell population, reflected by the disappearance of accessory cells, and with changes in cell density, morphology, and Na^+K^+-ATPase levels generally opposite to those induced by seawater adaptation (Foskett et al. 1983).

Mucous cells are often reported to be more abundant in freshwater-adapted fish. It has been proposed that an increased mucus layer in freshwater may be advantageous owing to the ability of mucus to bind sodium and chloride, impeding the outward passive diffusion of these ions and assisting their active absorption (Perry and Laurent 1993).

The increase in urine production following freshwater transfer is generally considered to result from an increase in the number of functioning nephrons, rather than an alteration of single nephron glomerular filtration rate (Henderson et al. 1985). However, an increase in the volume of Bowman's space and in glomerular diameter occurred in silvering eel following transfer to freshwater (Olivereau and Olivereau 1977), suggestive of an increased filtration rate. The increase in Bowman's space appeared within 2 days and was only transient (Olivereau and Olivereau 1977). It could therefore be related to the overshoot in urine production, which may occur 2–3 days following freshwater transfer (Talbot et al. 1989). The epithelial cell height and nuclear area increased, especially in distal and collecting tubules, also resulting here in an overshoot at day 2, and decreasing to freshwater levels after 5–10 days. No differentiation of new nephrons or mitotic activity was observed (Olivereau and Olivereau 1977).

2.2.2. Respiratory variables and acid–base status

Ventilatory frequency and amplitude rapidly decreased following freshwater transfer of adult Atlantic salmon (Maxime et al. 1990). The ventilatory flow reached a new steady state of 56% of the seawater value after 10 days. This lowering of the ventilatory flow was accompanied by a decrease in standard oxygen consumption to 80% of the seawater value. Since blood lactate concentration did not change, the decrease in oxygen consumption was probably due to a lowered oxygen requirement (Maxime et al. 1990).

The oxygen convection requirement (ventilatory flow – oxygen consumption) also decreased. This could be accounted for by a large increase in the oxygen extraction coefficient, probably due to increased branchial diffusive conductance for gases. In accordance with this view, arterial $Paco_2$ initially decreased in spite of a decrease in both ventilatory flow and water solubility of carbon dioxide (Maxime et al. 1990).

Blood pH, initially 7.94, increased to a maximal value of 8.43 at day 10, then decreased to a steady state value of 8.05. Maximal pH corresponded to maximal ionic perturbation and minimal $Paco_2$ and thus was a mixed metabolic–respiratory alkalosis (Maxime et al. 1990). After approximately 4 weeks, however, $Paco_2$ returned to seawater level and a purely metabolic blood alkalosis remained. The resulting rise in erythrocyte pH lead to an increase in the affinity of haemoglobin for oxygen, reinforced by the decrease in chloride concentration accompanying freshwater transfer (Maxime et al. 1990).

Thus, a rise in blood oxygen affinity as well as a decrease in energetic costs associated with ventilation and possibly basal metabolism occur following entry of adult salmon into freshwater. These may be important features in helping salmon to cope with the markedly increased energy expenditure required for upstream migration and may therefore be important parameters for natural selection.

3. Preadaptive changes

In the preceding chapter it has been shown that the transfer of salmonids between freshwater and seawater is accompanied by a number of physiological adjustments that may be energy- and time-consuming. During such a period of adaptation, a perturbation of homeostasis may occur which affects the fish's ability to avoid predators or capture prey (Brauner et al. 1994). Moreover, energy and metabolism may be directed towards adaptational changes rather than towards somatic growth, migration, or gonadal growth. Feeding behavior may be seriously impaired (McCormick 1994), which is particularly deleterious in species for which the residence period at sea is short, such as the Arctic char (Strand and Heggberget 1994; Finstad and Heggberget 1993). Most anadromous salmonids do therefore preadapt to these environmental changes, although to various degrees. The phenomenon of smoltification, which preadapts juveniles to seawater life, has been extensively studied and new aspects of adaptation are still being discovered. Numerous reviews dealing with smoltification are available (e.g., Folmar and Dickhoff 1980; Wedemeyer et al. 1980; McCormick and Saunders 1987; Hoar 1988; Bœuf 1993); therefore, only aspects directly related to migration and endocrinology will be discussed here. Preadaptation to freshwater life has on the other hand received little attention and present evidence for such adaptation will be discussed.

3.1. Preadaptation to seawater transfer

3.1.1. Common and differential features among salmonids

All salmonids begin their life in freshwater as stenohaline fish that usually die when exposed to seawater (McCormick and Saunders 1987). However, virtually all growing salmonids progressively develop some ability to osmoregulate in seawater, i.e., become euryhaline, due to a more favorable surface area:volume ratio for larger fish and (or) to a progressive development of hypoosmoregulatory mechanisms (McCormick and Saunders 1987). Such ontogenic changes are particularly rapid and complete in chum and pink salmon, which show high salinity tolerance shortly after emergence from the gravel (McCormick 1994; Varnavsky et al. 1993). Most anadromous salmonids, however, remain in freshwater for at least one year before migrating to the sea (McCormick 1994). This period in freshwater allows the parr to reach some critical size above which seaward migration may occur and may require from one to more than ten years depending on species and growth rate (McCormick 1994; Strand and Heggberget 1994).

In many stocks, seaward migration is associated with a period of rapid increase in seawater tolerance, occurring during smoltification or parr–smolt transformation (recently reviewed by McCormick and Saunders 1987; Hoar 1988; Bœuf 1993). Smoltification includes a number of morphological, behavioral, and physiological changes which are thought to be synchronized mainly by help of photoperiodic cues (Hoar 1988). In most smoltifying salmonids, the process is partly reversible and many of the changes will revert if the smolts are prevented from migrating to the sea (Hoar 1988). This process is commonly called desmoltification (Wedemeyer et al. 1980), although this term might by confusing since not all aspects of the transformation revert (Duston et al. 1991; Duston 1994). A new smoltification then generally occurs the next spring; smoltification thus appears like a seasonally occurring event. It may, however, proceed faster and to a greater degree for every year it occurs (Wedemeyer et al. 1980; Rydevik et al. 1989). There seems to be an interaction between photoperiod and ontogeny in regulating smoltification, in the way that some

seasonally occurring events may require some degree of development to appear and may be more pronounced in larger or older fish (McCormick and Saunders 1987). The relative importance of environmental influences and ontogeny seems to differ for different aspects of smoltification (McCormick and Saunders 1987). The degree of smoltification depends on species, stocks, and size/age of the fish (Conte and Wagner 1965; Prunet et al. 1989; McCormick and Saunders 1987; Rydevik et al. 1989; Johnston and Eales 1970). In species undertaking repeated migrations to the sea, it can be assumed that preadaptation may improve every year, at least to some maximal level. Such enhancement of smolt characters has been observed for silvering, loss of parr marks, hypoosmoregulatory capacity, and rheotactic behavior of Arctic char (Damsgård 1991; Schmitz 1992; Arnesen et al. 1995).

3.1.2. The interrelation between migration and smoltification

The time at which the young salmonid starts migrating is critical. Smoltification may develop over several years, especially in cold areas (Wedemeyer et al. 1980), and migration to the sea should not be initiated before a sufficient level of seawater tolerance is achieved. Migration to lower parts of the river, however, may be initiated much earlier if these areas are better for rearing than the spawning areas, as in glacial rivers or lakes (Murphy et al. 1997; Nilssen and Gulseth 1998). Moreover, since smoltification-associated seawater tolerance is limited in time, stocks originating from long rivers may have to start migration early enough to reach seawater before they have lost this capacity, whereas those originating from short rivers should start migrating close to their attaining maximal seawater tolerance. Thus, there must be some plasticity concerning the chronology of smoltification-associated changes. Indeed, silvering or development of salinity tolerance may increase before, at about the same time, or after downstream migration is initiated (Hasler and Scholz 1983). Migration to lower parts of the river, or to the estuary, may occur up to several years before complete seawater tolerance is achieved (Murphy et al. 1997; Nilssen and Gulseth 1998). The interrelation between the different events of smoltification is poorly understood (McCormick and Saunders 1987), but it is probable that timing of migration in relation to other changes has been subjected to strong natural selection. Understanding the interrelation between the different elements of smoltification may provide valuable information on the endocrine regulation of smoltification and migration.

As reported by Hasler and Scholz (1983) 25 years ago, "the mechanism of the seaward migration of salmon smolts has been the subject of much study, speculation, and argument," and any definite solution still does not exist. A number of changes associated with the parr–smolt transformation have been proposed to participate in triggering downstream migration and could therefore be expected to occur just prior to onset of migration. Increased buoyancy (Saunders 1965), decreased swimming ability (Smith 1982), preference for open areas (Iwata 1995), and decreased ability to maintain a visual position during evening twilight (Hasler and Scholz 1983) would favor passive downstream displacement of riverine fish. Increased exploratory behavior (Näslund 1990), downstream swimming activity (Lundqvist and Eriksson 1985), schooling behavior (Koike and Tsukamoto 1994), and decreased territoriality and aggressivity (Iwata 1995) would favor active downstream migration of both riverine and lacustrine smolts. In addition, a number of authors have suggested the existence of a direct connection between downstream migration and osmoregulatory dysfunction in freshwater caused by preparatory adaptation towards hypoosmoregulation (Thorpe 1984; McCormick and Saunders 1987). Atlantic salmon smolts may indeed have a net efflux of sodium through the gills in freshwater (Avella and Bornancin 1990) and may

suffer serious osmoregulatory imbalance in the absence of dietary intake of salts (Duston et al. 1991). Alterations in plasma or muscle ions during smoltification are, however, inconsistent (Folmar and Dickhoff 1980; McCormick and Saunders 1987) and migration may occur before smoltification is detectable according to seawater tests (Rottiers and Redell 1993; Nilssen and Gulseth 1998). This may therefore be an occasional rather than a general mechanism. McCormick and Saunders (1987) proposed that preparatory physiological changes could increase the osmoregulatory perturbation induced by environmental changes such as temperature and water flow, which are known to trigger migration. One could speculate that smoltification-associated changes in membrane lipids, which control cell permeability and compensation for temperature change (Hoar 1988), may be involved in an increased sensitivity to temperature. Virtanen and Forsman (1987) showed that a decrease in plasma chloride concentration and osmolality, as well as an increase in muscle moisture, occurred in wild Atlantic salmon smolts forced to swim against a constant flow, whereas no changes occurred in the parr. Thus increased water flow could cause water and ionic perturbation only in smolts. A close relation between smoltification-associated changes and onset of migration is suggested by the fact that larger fish, which smoltify earlier and faster than smaller ones, also migrate earlier. Such observation holds true for many species, including "single migrants" such as the Atlantic salmon (Bœuf 1993) and "repeat migrants" such as the Arctic char (T.G. Heggberget, Trondheim, unpublished data).

Another possibility for adjusting seawater entry with optimal seawater tolerance could be to let migration induce completion of preadaptive changes, since migration always precedes seawater entry. A few reports strongly indicate that migration may indeed play an important role in completing smoltification. Wild Atlantic salmon smolts, caught on the last few metres of their downstream migration and transferred to seawater within a few hours, were able to regulate plasma sodium concentration to freshwater level within 3 h (Chernitsky et al. 1993). This is significantly faster than reported in studies with hatchery-reared fish or wild fish retained in freshwater for a relatively long period of time (see Chernitsky et al. 1993). Chinook salmon released in the Columbia river and sampled 714 km from the point of release had gill Na^+K^+-ATPase activity levels 2.5 times greater than fish retained at the hatchery (Zaugg et al. 1985). Migration appeared as efficient as seawater transfer in increasing gill Na^+K^+-ATPase levels. Similar changes in gill Na^+K^+-ATPase activities were found in coho salmon and steelhead trout, in which enzyme activity level was shown to increase with time and migration distance (Zaugg et al. 1985). The degree of hypoosmoregulatory development prior to release affected the moment and rate of migration of the fish (Zaugg et al. 1985), suggesting that there may be some interaction between changes achieved prior to and during migration.

The mechanism involved in a possible migration-induced development of seawater tolerance remains open for debate. It can be assumed that environmental factors such as water flow, temperature, and light conditions, as well as physiological factors such as motor activity and stress level may be involved. These factors show great variations during river migration, in contrast to the stable situation usually met in hatcheries. Attempts to induce sustained elevated gill Na^+K^+-ATPase activity by altering water sources, changing holding environments and water flow have so far been unsuccessful (Zaugg et al. 1985). Sustained exercise during smoltification increased growth but not hypoosmoregulatory capacity in Atlantic salmon (Jørgensen and Jobling 1993). Endocrine changes associated with downstream migration include increased plasma growth hormone (McCormick and Björnsson 1994; Varnavsky et al. 1992), thyroxine (Youngson and Simpson 1984; Virtanen and Soivio 1985; Virtanen and Forsman 1987; Whitesel 1992; Youngson and Webb 1993; McCormick and

Björnsson 1994), and cortisol (Shrimpton et al. 1994; Mazur and Iwama 1993; McCormick and Björnsson 1994). These hormones are involved in many aspects of smoltification, including development of seawater tolerance (Hoar 1988; Sakamoto et al. 1993). Their effect during river migration is largely unexplored.

It is also possible that migration may delay the loss of salinity tolerance and thus increase the duration of the period when salmonids may successfully adapt to seawater. This could help smolts from long rivers reach seawater before the decrease in seawater tolerance occurs. In coho salmon, the greatest success in seawater adaptability occurred when fish were transferred to seawater at a time when gill Na^+K^+-ATPase activity had already begun to decline in hatchery-reared fish (Folmar et al. 1982). It can be speculated that migration inhibits this decline in the wild and allows gill Na^+K^+-ATPase activity to remain elevated until the fish reach seawater, at the optimal time for seawater entry. On basis of studies in Baltic salmon, Soivio et al. (1988) suggested that prevention of migration was an important factor in "releasing" the desmoltification process.

In conclusion, migration must be considered as an integrated part of smoltification, which may start at different points of the parr–smolt transformation. Therefore, studies concerned with the endocrinology of migration must necessarily take into account the endocrinology of smoltification.

3.1.3. Hormones and smolting

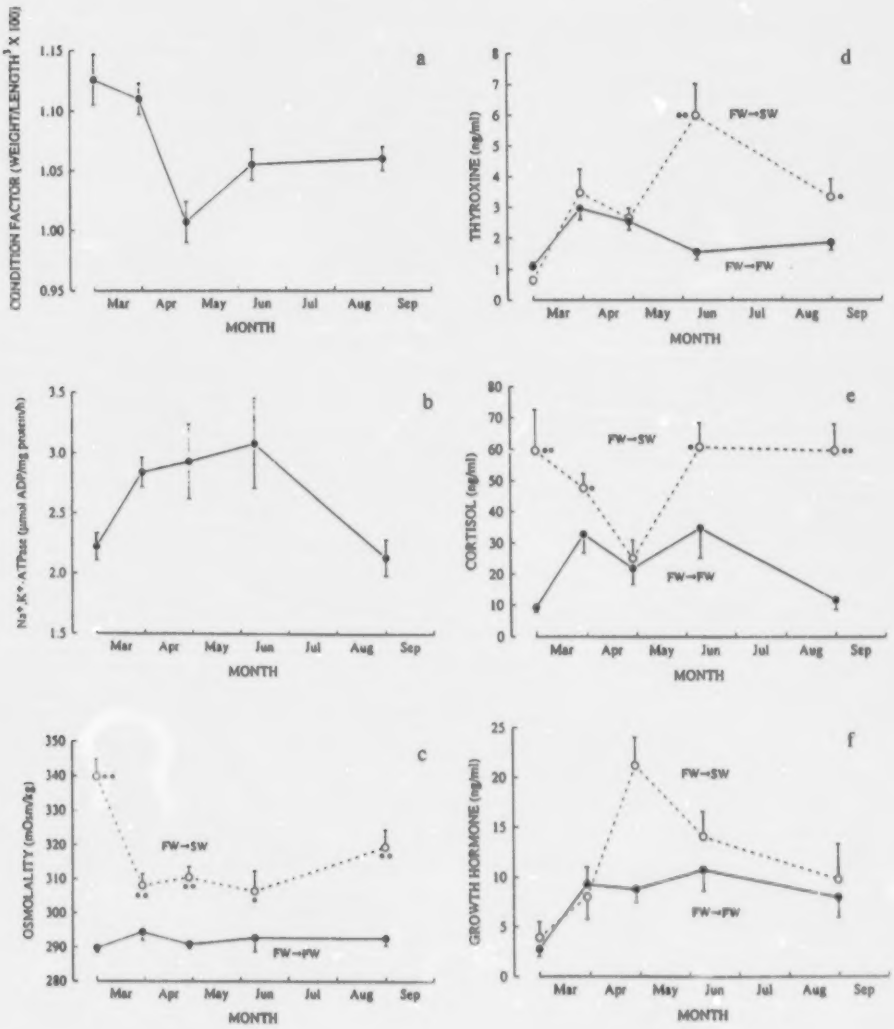
Smoltification is associated "with a general surge in endocrine activity that can be detected in most, if not all, of the endocrine glands" (Hoar 1988). In addition, smoltification is associated with changes in distribution, metabolism, clearance and effects of hormones, and with changes in the reactivity of endocrine glands to specific stimuli. It is therefore evident that endocrinology of smolting salmonids represents a complex field, where much care must be taken when interpreting the physiological meaning of isolated data, such as variations in the plasma concentration of hormones. Some examples are mentioned here, mainly to underline the complexity of this field. Details concerning the major hormones involved will be presented in Chapter 4.

Smoltification is accompanied by morphological signs of increased activity in most endocrine tissues, including the pituitary's TSH, growth hormone and prolactin cells, the thyroid gland, the interrenal cells, the pancreatic islets, the stannius corpuscles, and the caudal neurosecretory system (Bern 1978; Hoar 1988).

Increased plasma concentrations of insulin, calcitonin, thyroxine, triiodothyronine, sex steroids, growth hormone, somatolactin, cortisol, and catecholamines have been reported during smoltification (Hoar 1988; Bœuf 1993; Yamada et al. 1993; Rand-Weaver and Swanson 1993). In contrast, plasma prolactin concentration has been shown to decrease, sometimes after a transitional peak (Bœuf 1993). Endocrine changes may occur as early as the autumn preceding the year of smoltification, as described for plasma thyroxine (Hoar 1988). One may speculate that an increase in plasma thyroxine at that time could be involved in the autumn migration of parr, which in some river systems may represent up to 50% of the total annual production of migratory juveniles (Buck and Youngson 1982).

The effect of salinity on plasma concentrations of thyroxine, cortisol, and growth hormone changes during smoltification, as illustrated in Fig. 2. In this study, the effect of salinity change was distinguished from that of transfer by comparing hormone levels 24 h after transfer to seawater with values obtained in control fish transferred from freshwater to freshwater (Young et al. 1995). The level of smoltification is indicated by the condition

Fig. 2. Changes in (a) condition factor, (b) gill Na^+K^+-ATPase activity 24 h after transfer from freshwater to freshwater, (c) plasma osmolality, (d) thyroxine, (e) cortisol, and (f) growth hormone levels 24 h after transfer from freshwater to seawater (FW → SW) or from freshwater to freshwater (FW → FW) in yearling coho salmon. Each point represents mean ± S.E. (n = 8). *p < 0.05, **p < 0.01, compared to the FW → FW group. (Reprinted from Young et al. 1995. Circulating growth hormone, cortisol and thyroxine levels after 24 h seawater challenge of yearling coho salmon at different developmental stages. Aquaculture 136: 371–384. Copyright (1995) with permission from Elsevier Science.)



factor and the hypoosmotic regulatory capacity (see Fig. 2). Other changes in responsiveness during smoltification include increased cortisol response to stress or to ACTH (Barton et al. 1985; Young 1986), as well as increased thyroid gland responsiveness to TSH or increased water flow (Youngson et al. 1986; Bœuf 1993; Hoar 1988).

The effect of hormones may also change during smoltification. Exogenous administration of thyroxin, cortisol, growth hormone, or prolactin induced lipid mobilization in coho salmon parr but not smolts (Sheridan 1986).

The metabolism of hormones may show important variations. During smoltification of Atlantic salmon, the activity of thyroxine-5′-deiodinase, which is responsible for the conversion of thyroxine to triiodothyronine, increased first in the liver and the heart, later in the brain; the activity of thyroxine-5-deiodinase, which is responsible for the conversion of thyroxine to inactive rT3, increased in the brain (Yamada et al. 1993; Morin et al. 1993).

Finally, the distribution of hormones may change. In coho salmon, the concentration of thyroxine in the brain and the liver increased sharply and peaked in early smoltification, before plasma thyroxine increased (Specker et al. 1992). During the smoltification-associated surge in plasma thyroxine, the thyroxine content of muscle decreased (Specker et al. 1992). The fraction of thyroxine distributed to tissues decreased from 83% in early smoltification to 63% after smoltification (Specker et al. 1984). A decrease in the half-life of plasma cortisol and in corticosteroid receptor concentration and affinity in gill tissues was found in smoltifying coho salmon (Shrimpton et al. 1994).

3.2. Preadaptation to freshwater transfer

3.2.1. Experimental evidence

A clear indication of preadaptation to freshwater was provided by Potts et al. (1989). These authors compared the response to a direct transfer from seawater to freshwater of adult salmon and post-smolts adapted to seawater for 3 months. In adult Atlantic salmon, sodium uptake reached freshwater levels immediately after transfer. In contrast, sodium uptake in post-smolts did not reach this level before three days after transfer and sodium uptake immediately after transfer was only one-third of the rate of adult fish. Moreover, post-smolts were unable to maintain plasma sodium concentration as high as those of adult fish, even after 3 days. This strongly suggests that adult salmon preadapt to freshwater while still at sea. In marine fish, some sodium is taken up in exchange with hydrogen ions for acid–base regulation. It is likely that the capacity of the Na^+-H^+-antiport system increases as the salmon approach freshwater prior to their upstream migration (Potts et al. 1985; Potts et al. 1989).

There are several indications that salmonids may loose some of their hypoosmoregulatory capacity around the time of upstream migration. During the early stages of final gonadal maturation, salmon forced to remain in seawater may experience osmoregulatory difficulties, e.g., substantial increases in sodium levels and osmolality, as shown in coho salmon (Sower and Schreck 1982). When forced to remain in seawater, these fish showed a higher mortality rate (Sower and Schreck 1982). Increasing mortality occurred similarly in Arctic char kept in seawater beyond the time of normal upstream migration (Arnesen and Halvorsen 1990). The fish were transferred to seawater during the period of downstream migration. After 40–45 days, some mortality appeared, and total mortality reached 30% at 79 days after transfer (Arnesen and Halvorsen 1990). When caught in the river, upstream migrating salmon may not survive direct transfer into seawater,

as shown in sockeye and chum salmon (Fontaine 1975). A progressive decline in seawater tolerance was observed in wild Arctic char exposed to seawater 7, 10, and 21 days after freshwater entry (Nilssen et al. 1997). In these cases, however, it is unclear whether the loss of seawater tolerance was timed by an endogenous clock or was induced by freshwater entry.

The functional significance of the decrease in hypoosmoregulatory capacity is uncertain. It probably represents an important adaptation, or preadaptation, to the river migration and freshwater stay. Decreases in the amount of chloride cells and their leaky junctions decrease the ionic permeability of the gills, favoring osmoregulation in freshwater (Evans 1993). Moreover, a decrease in the number of chloride cells increase the respiratory area, favoring gas transfer (Bindon et al. 1994a). In addition, if the decrease in hypoosmoregulatory capacity occurs in seawater, it could motivate upstream migration. There is evidence that salmonids normally enter freshwater before a significant decline in hypoosmoregulatory capacity occurs. Wild anadromous Arctic char from the Spitsbergen Island were shown to still have a good hypoosmoregulatory capacity 7 days after they returned to freshwater (Nilssen et al. 1997). However, if fish lack the cues necessary for homing, e.g., hatchery-reared fish escaped from net pens, they may hypothetically remain in seawater until their hypoosmoregulatory capacity declines. This, however, remains to be shown.

Adult salmonids seem to preadapt for the high motor activity associated with upstream river migration. The metabolism of white muscle in maturing rainbow trout has been shown to become increasingly aerobic, with an increased capacity for fatty acid utilization and a decreased glycolytic capacity (Kiessling et al. 1995). Mature salmon recover faster than juveniles following intense exercise (Woodhead 1975).

Evidence of preadaptation of salmonids to orientation during river migration is discussed in Hasler and Scholz (1983). Before the upstream-migration phase, it appears that salmon cannot distinguish their home stream odor, whereas at about the time they reach the coast and begin migrating upstream, they can. If coho salmon are released into a river during the open water portion of their migration, they remain predominantly stationary, whether the river contains home water or not. In contrast, during the period of river migration, the fish move upstream in the presence of home water and downstream in its absence. Hence, the river part of homing of coho salmon appears to result from a preadaptation making the fish sensitive to imprinted or genetically memorized odor and prone to upstream migration in the presence of the scent (Hasler and Scholz 1983). Quantitative changes in salmon GnRH and in an olfactory-system related protein, N24, apparently correlated to homing behavior, have been evidenced in kokanee salmon, *O. nerka* (Ueda et al. 1995). It is still uncertain whether there is a significant adaptation of the vision of the fish to upstream migration. No changes in retinal pigments were found in Atlantic salmon smolts in the sea and adult upstream migrants (Woodhead 1975). However, large numbers of putative ultraviolet (UV)-sensitive cones were recently identified in the retina of sexually mature Pacific salmon (Beaudet et al. 1997). These cones normally disappear from the main retina around the time of smoltification and downstream migration, as shown in rainbow trout, brown trout, Atlantic salmon, and sockeye salmon (see ref. in Beaudet et al. 1997), and their reapparition in sexually mature individuals may indicate that they play some role in the homing migration (Beaudet et al. 1997; Kunz et al. 1994). Studies are needed to time more precisely the moment when they reappear and to evaluate the significance of such changes in UV photosensitivity. Thyroxine may trigger both the disappearance and reappearance of these cones, but its physiological significance in this process has not been demonstrated (Browman and Hawryshyn 1994; Beaudet et al. 1997).

3.2.2. Putative relation with desmoltification

Smoltification is typically a reversible process, at least to some extent, and smolts kept in freshwater revert to a parr-like condition after a few weeks or months. Exceptions to this rule include pink and chum salmon, as well as some stocks of Atlantic salmon that do not seem able to readapt to freshwater (Baggerman 1960; Bœuf 1993; Rottiers 1994). In other stocks of Atlantic salmon, some smoltification-associated changes such as increased growth potential and new feeding habits are retained in post-smolts kept in freshwater (see Duston et al. 1991). In most salmonids, however, "desmoltification" includes a loss of silvery coloration, an increase in condition factor and fat deposits in muscle, liver, gut, and gills, and a change in fat composition towards the freshwater pattern (Lundqvist and Eriksson 1985; Li and Yamada 1992). It also includes a number of changes that would probably induce upstream river migration and possibly homing in a salmonid present in coastal seawater: a decrease in seawater tolerance, as shown by decreasing gill Na^+K^+-ATPase activity and increased ionic and respiratory perturbation and mortality following seawater transfer, a decrease in salinity preference, a change from downstream to upstream orientation, and an increase in olfactory sensitivity (Conte and Wagner 1965; Baggerman 1960; Stagg et al. 1989; Schmitz 1992; Lundqvist and Eriksson 1985; Morin et al. 1994).

One may wonder whether desmoltification may indeed induce upstream migration in a salmonid undertaking short seasonal migrations to the sea. One interesting candidate would be the Arctic char. In Northern Norway and Svalbard, Arctic char grow very slowly in freshwater lakes, but once they have reached a minimal size, they migrate to the sea in early summer, where ample food resources lead to fast growth (Strand and Heggberget 1994; Finstad and Heggberget 1993). Seasonal feeding migrations to the lower parts of the river or to the estuary may also occur in fish smaller than the threshold for developing full seawater tolerance (Nilssen and Gulseth 1998). Although temperature and food availability are still optimal in late summer, Arctic char invariably return to their winter habitat after feeding in coastal areas for 3–8 weeks (see Finstad et al. 1989; Finstad and Heggberget 1993; Berg and Berg 1993; Nilssen et al. 1997). Finstad et al. (1989) showed that seawater tolerance of Arctic char was high in summer and low in autumn and winter and suggested that return migration could be "triggered by photoperiodic changes, accompanied by osmoregulatory adjustments in favor of the expected freshwater life." Recent studies have demonstrated that Arctic char smoltify (Arnesen et al. 1992; Staurnes 1993; Damsgård 1991; Arnesen et al. 1995; Halvorsen et al. 1993) and an obvious question seems therefore to be: could the return migration of Arctic char be associated with desmoltification?

To assess whether desmoltification and upstream migration are associated in Arctic char, one should first study the duration of the smoltification–desmoltification cycle in Arctic char under natural conditions. In Svalbard, small Arctic char, older than 2 years but too small to acquire complete seawater tolerance, migrate to lower parts of the river to feed for a period of 5–10 weeks, then return to their lake of residence (Gulseth and Nilssen, in press). Thus this behavioral aspect of the smoltification–desmoltification cycle has a duration of 5–10 weeks under such conditions. The few published data on the duration of the changes in seawater tolerance indicate a similar duration of that aspect. If downstream migrating Arctic char are retained in freshwater for 16 days, they still possess excellent seawater tolerance (Halvorsen et al. 1993), suggesting that the fish keep their smolt status for at least 16 days beyond the time of downstream migration when kept in freshwater. When Arctic char were kept in seawater (35‰) after normal time for seawater entry, some mortality appeared after 40–45 days and total mortality was 30% at 79 days after transfer (Arnesen

and Halvorsen 1990). The normal seawater stay of the studied population was on average 42 days, which corresponds to the duration of full seawater tolerance (Arnesen and Halvorsen 1990). However, desmoltification is highly sensitive to temperature, in the way that increasing temperatures accelerate desmoltification (Wedemeyer et al. 1980; Duston et al. 1991), thus local temperature changes should be taken into account. Under natural conditions, temperature increases during summer and an acceleration of desmoltification may thus occur in late summer. Desmoltification may therefore typically occur when seawater temperature still is optimal for feeding and growth, which is the case as Arctic char return to their freshwater winter habitat.

If upstream migration of coastal salmonids is to be induced by desmoltification, then behavioral changes such as increased freshwater preference and homing behavior must occur in seawater. These aspects have received little attention, but it is reasonable to assume that they anticipate loss of seawater tolerance, which has been shown to still be good one week after freshwater entry of Arctic char (Nilssen et al. 1997). The regulation of the different elements of desmoltification is best studied for the loss of seawater tolerance. Sockeye, coho, or chinook salmon do not lose their seawater tolerance when kept at salinities of 10–20‰ (Wedemeyer et al. 1980). Arctic char, however, show a decline in seawater tolerance even in 35‰ seawater (Arnesen and Halvorsen 1990), suggesting interspecific differences in the regulation of desmoltification. If Pacific or Atlantic salmon are transferred to seawater at an inappropriate time, they may also desmoltify in full seawater, giving rise to the "parr-revertants," which under natural conditions probably return to freshwater (Folmar et al. 1982). In Baltic salmon, a salinity of 5–6‰ did not inhibit desmoltification (Soivio et al. 1988). Thus inhibition of desmoltification in most Pacific or Atlantic salmon, as shown by seawater tolerance, seems to depend upon both a high salinity and an optimal period of transfer to seawater. The Arctic char in northern Norway seems to be at one extreme, with desmoltification occurring even in full seawater. At the other extreme, we find pink and chum salmon, as well as some stocks of Atlantic salmon (Baggerman 1960; Bœuf 1993; Rottiers 1994), in which desmoltification is inhibited even in freshwater.

This leads us to speculate on the evolution of the smoltification cycle. The Arctic char belongs to the genus *Salvelinus*, which is considered the most primitive of the anadromous salmonids (McCormick 1994). The brook trout, another *Salvelinus*, similarly undertakes short migrations (2–4 months) to the sea during the summer season (McCormick et al. 1985). The timing and duration of seaward migration is more variable in southern populations than in northern populations of brook trout. Anadromous stocks start migrating at a small size, but only migrate as far into the estuary as their seawater tolerance allows them to, giving rise to a size-dependent migration (McCormick et al. 1985). Seasonal variations in seawater tolerance and plasma osmolality, chloride, glucose, and thyroxine similar to those observed at the time of smoltification in other salmonids have been reported in brook trout (Audet and Claireaux 1992). However, anadromous and nonanadromous stocks show similar variations and these are much smaller than in smoltifying Atlantic salmon (McCormick et al. 1985). McCormick et al. (1985) suggested that seasonal changes in thyroid hormones in the brook trout could be related to functions other than migration, such as increased feeding or somatic growth.

Systematic analysis and fossil evidences indicate a freshwater origin of salmonids, with *Salvelinus* being more primitive than *Salmo*, which in turn is more primitive than *Oncorhynchus* (McCormick 1994). Although this view is challenged (see e.g., Thorpe 1988), one may take it as a starting point and speculate that anadromous salmonids originally took advantage of seasonally occurring metabolic and behavioral changes to

undertake short migrations into lower parts of the river, estuaries and (or) coastal areas. Natural selection progressively favored the fish that were most strongly preadapted to seawater life, i.e., those with the greatest amplitudes of changes and the best temporal coordination of specific changes. Different levels of "smoltification" developed. This natural selection may have been particularly strong in northern areas, due to the short summer season, explaining the stronger smoltification of Arctic char as compared to the more southern brook trout (Arnesen et al. 1992; McCormick 1994; Dempson and Green 1985). Progressively, increasing levels of preadaptation may have allowed smaller and smaller fish to successfully migrate towards the sea. There is indeed a clear relation between genus and size at seawater entry among anadromous salmonids (see McCormick 1994).

Another important step in the evolution of anadromy was probably the ability to remain in seawater for a longer period of time. By doing so, salmonids became able to reach more distant areas and to undertake the upstream migration at a greater size. This may have represented a particularly strong selective advantage in species migrating to the sea at a small size, since a short seawater stay restricted their ability to undertake upstream migration and made them more vulnerable to predation in the estuary. One may speculate that the development of a regulatory pathway by which seawater entry was able to inhibit desmoltification offered this opportunity. Whereas most *Salvelinus* species may not have developed this regulatory pathway, most *Oncorhynchus* and *Salmo* species have. The level of salinity inhibiting desmoltification and the level of smoltification at which a high salinity is able to inactivate desmoltification depends on species or stocks (see above). One could speculate that the extent of the desmoltification process is also species- and stock-dependent. In Atlantic salmon, growth and feeding habits acquired during smoltification seem to remain even in freshwater, whereas most other smoltification-associated changes revert (Duston et al. 1991). In other species or stocks, the pattern could be different. The term "desmoltification" might thus not be appropriate and will perhaps be replaced by more specified changes as more knowledge in this field is gained.

The regulation of desmoltification is complex and the effect of salinity would most probably have been added to existing pathways. Present knowledge suggests that desmoltification is affected by water temperature (Wedemeyer et al. 1980; Duston et al. 1991), salinity (Folmar et al. 1982; Clarke et al. 1981; Soivio et al. 1988), day-length (Soivio et al. 1988; Iversen 1993), fish density (Soivio et al. 1988), migration (Soivio et al. 1988), and level of smoltification at seawater entry (Folmar et al. 1982). It is suspected that the local water quality, in particular its content of different salts, could play some role in regulating desmoltification as well. The stocks of Atlantic salmon which do not desmoltify when kept in freshwater (see Bœuf 1993) should be reared in different water qualities to test this hypothesis.

One can finally speculate that upstream migration in salmonids who spend at least one year at sea may be associated with a reactivation of some kind of desmoltification process, for the following reasons. First, the development of upstream river orientation, increased olfactory sensitivity, and decreased hypoosmoregulatory capacity are features which are common to both desmoltification and upstream migration of adults (see above). Second, desmoltification is often associated with sexual maturation in male Baltic salmon (Fängstam et al. 1993). Finally, seawater-adapted Atlantic salmon do show seasonal changes in osmoregulatory parameters such as Na^+K^+-ATPase activity in both gills and intestine (Talbot et al. 1992; Gjevre 1993), as well as in the plasma concentration of the freshwater-adapting hormone prolactin (Andersen et al. 1991a).

The endocrine regulation of desmoltification has apparently received little attention, if

any. Desmoltification is often associated with sexual maturation in male Baltic salmon (Fängstam et al. 1993), indicating some relation between sex hormones and desmoltification. Secondary peaks in plasma thyroxine and triiodothyronine (Prunet et al. 1989; Bœuf et al. 1989), growth hormone (Prunet et al. 1989), prolactin (Young et al. 1989), and cortisol (Young et al. 1989) have been reported during the period of decreasing levels of gill Na^+K^+-ATPase activity in Atlantic or coho salmon. However, the significance of these changes is unknown.

To conclude, the concept of "desmoltification" needs to be reconsidered. This process seems to be under complex regulation and to be another important element of the plasticity of salmonids. The putative homology between desmoltification and upstream migration should be tested, since desmoltification would then provide a convenient model for studying the regulation of upstream migration. This approach may cast light on the largely unexplored determinants of upstream migration. In particular, if we adopt the opposite view that salmonids originated in seawater, as supported by Thorpe (1988), then upstream migration and "desmoltification" would be the start of a cycle leading marine fish to temporally enter freshwater and downstream migration and "smoltification" would be the end of that cycle. Then the "early decision to smoltify," which occurs some 9 months before the spring migration and includes a rise in plasma thyroxine and a high appetite and growth rate (see Metcalfe and Thorpe 1990; Hoar 1988), could be a remnant of the first "desmoltification." This term would then obviously need to be changed.

4. Endocrinological aspects

4.1. Thyroid hormones

Increased thyroid activity during smoltification and downstream migration of anadromous salmonids has been demonstrated by the histological appearance of the thyroid follicles (Hoar 1939), by radioiodine uptake studies (Baggerman 1960), and by radioimmunoassay of plasma thyroid hormones (Dickhoff and Sullivan 1987). However, efforts to ascribe causality to thyroid hormones for migration, smoltification, or salinity transfer have been only partially successful. One reason for the difficulties encountered may lie in the recent discovery that each tissue is able to regulate its content of thyroxine (T_4) and triiodothyronine (T_3) and that major changes in the distribution of thyroid hormones occur during the development of salmonids (Specker et al. 1984; Eales 1985; Specker et al. 1992). Changes in thyroid hormone production or plasma content give, therefore, no precise indication about thyroid activity at the receptor level. The complexity of thyroid activity is also illustrated by the biphasic effect of hormone treatments (Dickhoff and Sullivan 1987).

Present knowledge on general thyroid physiology in fish will first be presented. Then, the specific role of thyroid hormones in migration, smoltification, and salinity transfer will be discussed.

4.1.1. General aspects of thyroid physiology in fish

4.1.1.1. Production of thyroid hormones

Thyroxine (3,5,3′,5′ tetraiodothyronine, T_4) is synthesized by thyroid follicles, which in salmonids are scattered throughout the subpharyngeal and parapharyngeal area (Gorbman 1969; Bœuf 1987). T_3 (3,5,3′ triiodothyronine), in contrast, seems to derive exclusively from peripheral outer-ring deiodination of T_4 (Eales 1990). For comparison, about 20% of T_3 production occurs in the thyroid gland in mammals (West 1990).

As in mammals, T_4 production in teleosts is stimulated by thyroid stimulating hormone (TSH) released from the pituitary (Leatherland 1988). Plasma T_4 is consistently elevated by injection of mammalian TSH, whereas elevation of plasma T_3 is less consistent (Brown et al. 1978; Specker and Schreck 1984; Leatherland 1988).

In contrast to mammals, the pituitary–thyroid axis of several teleost species, including the rainbow trout, may be under dominant inhibitory control by the hypothalamus (Leatherland 1982). Somatostatin, epinephrine, and norepinephrine are all thyrotropin inhibiting hormone (TIH) candidates (Eales et al. 1986; Leatherland 1988). The few studies specifically examining the effect of thyrotropin releasing hormone (TRH) in teleosts are contradictory (Leatherland 1988). Thyroid activity has been reported to decrease, increase, or remain at the same level after treatment with mammalian TRH (Leatherland 1988). Biphasic effects may explain these results. Moreover, considerable differences in sensitivity to exogenous TRH have been reported, the Arctic char responding to intraperitoneal injection of doses 1000 times lower than the rainbow trout (Eales and Himick 1988). These differences in sensitivity could be related to anatomical differences influencing the delivery of TRH to the pituitary (Leatherland 1982).

In the Arctic char, TRH induced an increase of plasma T_4 from 2 ng·mL^{-1} to 8 ng·mL^{-1} at 1–6 h after injection, levels being back to normal by 24 h (Eales and Himick 1988). Thus plasma T_4 may change within a few hours following appropriate stimulation and T_4 is rapidly cleared from plasma as compared to mammals. For comparison, the half-life of

circulating T_4 is 6.5 days in human (West 1990). The increase in plasma T_4 is modest following TRH injection as compared to that seen following TSH injections, which suggests that the thyrotroph reserve of TSH available for immediate release may be limited in salmonids (Eales and Himick 1988).

The responsiveness of salmonids to TSH or TRH injection may decrease in fasted fish (Eales 1985; Eales and Himick 1988). It may increase with fish size (Chan and Eales 1976) and during smoltification (Specker and Schreck 1984; Lin et al. 1985).

Daily rhythms in plasma T_4 concentration have been evidenced in rainbow trout (Eales et al. 1981; Reddy and Leatherland 1994; Boujard et al. 1993; Gomez et al. 1997), brook trout (White and Henderson 1977; Audet and Claireaux 1992) and Atlantic salmon (Youngson et al. 1986). In rainbow trout starved for at least 3 days, the diel variations disappeared (Brown et al. 1978; Eales et al. 1981). In fed trout, both feeding time and photoperiod seem to regulate the daily rhythm of plasma T_4 concentration (Reddy and Leatherland 1994; Spieler 1992).

Plasma levels of thyroid hormones may be affected by stress. Changes have been reported in rainbow trout following transport, netting, and confinement, and after physical injury caused by a needle during blood sampling or injection (see Pickering 1993).

4.1.1.2. Blood transport

Thyroid hormones are lipophilic molecules and are transported in plasma bound to proteins. Thyroxine binding globulin (TBG), which is the main transport protein for thyroid hormones in mammals, has not been detected in teleosts (Bœuf 1987). In brook trout, thyroid hormones are bound to several protein fractions (prealbumin-like, albumin-like, and β-globulin-like proteins) (Falkner and Eales 1973). In rainbow trout and Arctic char, the free fractions of plasma T_4 and T_3 are approximately 0.2 and 0.1%, respectively, vs. 0.03 and 0.3%, respectively, in mammals (Eales and Shostak 1985; West 1990). The relatively low binding of T_4 in fish plasma explains its higher plasma clearance (see above), probably closer to that of mammalian T_3 than mammalian T_4 ($t_{1/2}$ of 1.3 and 6.5 d, respectively). Therefore, a finer regulation of thyroid activity through changes in plasma T_4 is probably possible in fish, as compared to mammals. Moreover, although total T_4 concentration is 40 times lower in Arctic char than in humans, the free T_4 (F_4) concentration is only 4 times lower in Arctic char. The concentrations of free hormones are close for T_4 and T_3, 5 and 3 pmol·L^{-1}, respectively, in Arctic char (Eales and Shostak 1985).

Variations in free fractions (F) of thyroid hormones could explain aspects of environmentally modified thyroid hormone metabolism (Eales and Shostak 1986). Percent F_4 and percent F_3 in Arctic char plasma *in vitro* increased with pH within physiological range (7.0–7.8). Percent F_4 and percent F_3 also increased with temperature *in vitro* and *in vivo*, when Arctic char were acclimated at 5, 13, and (or) 20°C. However, the physiological consequence of such changes remains uncertain until we know if tissue TH-cytosolic binding sites also change their affinity to T_3 and T_4 with temperature (Eales and Shostak 1986). The effect of temperature on receptor affinity has been studied in the lamprey, for which temperature changes during either laboratory maintenance or incubation (10 or 20°C) had no significant influence on hormone binding (Eales 1985). Bœuf et al. (1989) showed that during smoltification of Atlantic salmon, percent F_4 and F_3 levels in plasma remained stable. The period of sampling extended from February to July, when temperature increased from 2 to 14°C. Only at one single sample time (24 June), coinciding with decreasing gill Na^+K^+-ATPase, was there a decrease in the free fraction of hormones (Bœuf et al. 1989).

4.1.1.3. Distribution to tissues

It has recently become evident that plasma content of thyroid hormones does not necessarily reflect tissue content. Morin et al. (1989) showed a discrepancy between a high thyroidal activity, as judged by histological criteria (epithelial cell height, follicular eccentricity), and plasma T_3 and T_4 concentrations, during photoperiodically induced smoltification of juvenile Atlantic salmon. Scholz et al. (1985) showed that during parr–smolt transformation of steelhead trout, injected radiolabelled T_3 was rapidly taken up by the brain, lowering plasma level. In smoltifying coho salmon, the brain and liver content of T_4 increased significantly and reached its highest level at the beginning of smoltification, before plasma T_4 increased (Specker et al. 1992). The fraction of T_4 distributed to tissues decreased from 83% in early smoltification to 63% after smoltification in coho salmon (Specker et al. 1984).

Increased plasma concentration of T_4 could reflect in part decreased flux of the hormone from blood to tissues (Bern and Nishioka 1993). Indeed, the T_4 content in muscle decreased during the smoltification-associated rise in plasma T_4 in coho salmon (Specker et al. 1992).

Specker et al. (1984) suggested that a shift in the tissues to which T_4 is distributed occurs during smoltification of coho salmon. Kinetic parameters indicated that T_4 was mainly distributed to slow equilibrating tissues, possibly muscle, skin, or fat during smoltification, and to fast equilibrating tissues, possibly liver and kidney, after smoltification (Specker et al. 1984).

4.1.1.4. Deiodination

Conversion of T_4 to T_3 by outer-ring deiodination has been evidenced in liver, muscle, gill, kidney, brain, and heart of salmonids (Eales et al. 1993; Morin et al. 1993). According to Morin et al. (1993), the liver is the main source of plasma T_3. Receptor-bound T_3 seems to be derived primarily from plasma T_3 in kidney, mainly from intracellular T_4 to T_3 conversion in gills, and about equally from plasma and intracellular sources in liver (Eales et al. 1993). Local deiodination of T_4 is also an important source of T_3 in the brain of smoltifying Atlantic salmon (Morin et al. 1993). Increased plasma T_4 may therefore lead to increased tissue T_3 without plasma T_3 being significantly changed. Moreover, decreasing plasma T_4 as a result of increasing T_4 flow into tissues could be associated with increased tissue and plasma T_3. This could explain why a peak in plasma T_3 sometimes occurs after the peak in plasma T_4 (Bœuf 1993).

At least two isoenzymes are involved in outer-ring deiodination of T_4 (Eales et al. 1993); one high-affinity (Km range 0.1–1 nM), propyl-thiouracil (PTU) sensitive, present in several tissues, possibly producing T_3 for local use; the other low-affinity (Km $\geq$10 nM), PTU-insensitive, in liver and kidney, possibly producing T_3 for systemic use. In view of the changes in tissue distribution occurring during smoltification (see above), the different coefficients of affinity and sensitivities of the two deiodinase systems are of interest. Enzymes are considered to operate at substrate levels approximating their Km values (Eales et al. 1993) and the high affinity deiodinase is therefore adapted to operate at the T_4 levels that can be expected intracellularly when plasma T_4 is at base level (2.5–5 nM) (Specker et al. 1984; Eales et al. 1986; Eales and Himick 1988). The low affinity deiodinase can be expected to be maximally active during peaks of plasma T_4, such as that occurring during smoltification (about 50 nM in coho salmon (Dickhoff and Sullivan 1987)). Its localization in liver and kidney suggests that the possible switch occurring from a distribution of T_4 to muscle, skin, and fat prior the T_4 peak, to liver and kidney after the peak (Specker et al. 1984), may be due to a marked effect of thyroid hormones on the latter organs at the time of

the plasma T_4 peak, possibly inducing the synthesis of thyroid receptors or other intracellular thyroid hormone binding proteins.

Whereas plasma T_4 shows large fluctuations in fish, plasma T_3 is generally maintained relatively constant (Hoar 1988). The liver iodothyronine deiodinase systems act in a coordinated manner to maintain this constancy of plasma T_3. Administration of exogenous T_3 to rainbow trout resulted in reduced conversion of T_4 to T_3, increased conversion of T_4 to inactive rT_3 (3,3',5'-triiodothyronine), and increased conversion of T_3 to T_2 (3,3'-diiodothyronine). Since the half-life of hepatic deiodinase is short, a fine regulation of T_3 levels is possible (Eales et al. 1993). Response to a 4-fold greater dietary T_4 challenge was restricted primarily to a depression of T_4 deiodinase activity in the liver and the kidney (McLatchy and Eales 1993).

Deiodination of thyroid hormones in fish is sensitive to several factors other than plasma T_3 and T_4. An adequate nutritional state, a testosterone-induced anabolic state, increased temperature, growth hormone, and cortisol all increase deiodination, whereas starvation or estradiol decrease deiodination (Eales 1985; Vijayan et al. 1988; Cyr et al. 1988; Leloup and Lebel 1993). Injection of ovine prolactin has been shown to increase T_4 to T_3 conversion in coho salmon and rainbow trout, but this effect may be a nonspecific growth hormone-like effect that salmonid prolactin may not have (Leatherland 1988). Dietary amino acid composition also affects deiodination (Riley et al. 1993). During smoltification of Atlantic salmon, T_4-5'-deiodinase activity increased first in liver and heart and later in brain, whereas activity levels in gill or skeletal muscle remained constant (Morin et al. 1993). Brain T_4-5-deiodinase, which produces inactive rT_3, showed a progressive increase in activity during smoltification (Morin et al. 1993). The significance of these changes is at present unknown.

Bœuf et al. (1989) observed two clearly pronounced T_4 peaks but only fluctuations in plasma T_3 in smoltifying Atlantic salmon. Since a decrease in plasma T_3 was coincident with the two major surges in plasma T_4, the authors suggested that a decrease in T_4 to T_3 conversion may have contributed to the T_4 peaks. Similarly, Morin et al. (1993) observed a peak in plasma T_3 1–2 weeks before the T_4 peak during photoperiodically induced smoltification of Atlantic salmon. In contrast, plasma T_3 peaked after plasma T_4 in smoltifying coho salmon, which led Dickhoff and Sullivan (1987) to suggest that an activation of the 5'-deiodinase system in response to the increased plasma T_4 levels in early smoltification may occur.

One could speculate that a differential regulation of the two isoenzymes may contribute to the relative changes in plasma T_3 and T_4, as well as the changes in tissue flux, which occur during smoltification. The existence of such a differential regulation is suggested by their different sensitivities to PTU. The threefold increase in T_4 secretion occurring in early smoltification (Specker et al. 1984) may first be associated with a rapid deiodination to T_3 through the high affinity deiodinase, causing an increase in plasma T_3 while plasma T_4 changes little. "Some factor," possibly induced by T_3 activity or other hormones, could then inhibit specifically the high affinity deiodinase, inducing decreased plasma T_3 while plasma T_4 increases. At sufficient levels, T_4 could be deiodinated by the low-affinity deiodinase, possibly causing the shift of flow of T_4 to liver and kidney, the decrease in plasma T_4, and the second T_3 peak.

4.1.1.5. Thyroid hormones receptors

Thyroid hormones, like steroid hormones, retinoids and vitamin D, easily diffuse through the plasma membrane and bind to intracellular receptor proteins. This receptor–

hormone complex regulates the transcription of specific genes, generally in association with other gene regulatory proteins (Alberts et al. 1994). Such a dependency to other regulatory proteins allows for a wide spectrum of effects, specific to each tissue and each physiological state. In fish, injection of thyroid hormones may increase the activities of cytochrome oxidase and glycerophosphate dehydrogenase and decrease the activities of mitochondrial Mg^{2+}-ATPase, cytosolic and mitochondrial malate dehydrogenase (Peter and Oommen 1993). Thyroid hormones may also induce the synthesis of a "somatomedin-like" substance (see Darling et al. 1982).

Thyroid hormone receptors have been found in liver, gill, kidney, and brain of salmonids (McCormick 1995; White et al. 1990). Hepatic receptors show a 10-fold greater affinity for T_3 than T_4. This is similar to mammalian receptors, suggesting that the receptor molecule has been highly conserved during evolution (Darling et al. 1982; Eales 1985).

The maximal binding capacity of thyroid receptors in liver nuclei was depressed with the size–age of rainbow trout (Eales 1990). However, receptor regulation is probably of little importance for regulation of thyroid status as compared to 5′-deiodinase regulation (Eales 1990).

4.1.2. Possible involvement of thyroid hormones in smoltification

Thyroid hormones have been implicated in the control of salmonid smoltification for over half a century, yet their precise role remains poorly understood (Dickhoff and Sullivan 1987). Recently, Eales (1990) stated that "despite increasing sophistication in evaluation of the thyroidal status, the role if any, of the thyroid hormones during this critical phase of the salmonid life cycle is obscure." In view of the complex regulation of thyroid status depicted above, existing methods for evaluation of the thyroidal status during smoltification may in fact not yet be sophisticated enough to clearly understand the exact role of thyroid hormones.

Evidence for a role of thyroid hormones is provided by the observation of important changes in thyroid physiology during smoltification and by studies on the ability of thyroid hormones to regulate some aspects of smoltification. Studies supporting these two lines of evidence will be reviewed successively.

4.1.2.1. Changes in thyroid physiology during smoltification

A number of changes in thyroid physiology have been demonstrated during the parr–smolt transformation, including the following:

- histological signs of activation of the thyroid gland, as shown by a columnar type epithelium, vacuolization, and loss of colloid from the follicular lumen (Hoar 1939), elliptic follicles (Morin et al. 1989), development of endoplasmatic reticulum, Golgi system and increased number of secretory vesicles (Nishioka et al. 1982);
- increased iodide uptake (Baggerman 1960);
- increased responsiveness of the thyroid gland to bovine TSH (Specker and Schreck 1984), exposure of the fish to increasing current velocity (Youngson and Simpson 1984; Youngson et al. 1986) or to novel water or environment (Lin et al. 1985);
- a fourfold increase in T_4 secretion rate (Specker et al. 1984);
- plasma T_4 surge(s) occurring some weeks to some days prior to maximal seawater tolerance, reaching 3–7 fold pre-surge level (Dickhoff et al. 1978; Bœuf 1993);

- plasma T_3 surge(s) prior to and (or) after the T_4 surge, reaching about 2-fold pre-surge level (Dickhoff et al. 1982; Bœuf 1993);
- an increase in T_4 to T_3 conversion in liver, heart, and brain (Yamada et al. 1993; Morin et al. 1993);
- an increase in T_4 to rT_3 conversion in the brain (Morin et al. 1993);
- changes in tissue distribution of T_4 (Specker et al. 1984, 1992).

Bœuf et al. (1989) demonstrated in Atlantic salmon that the free fraction of T_3 and T_4 was not altered during smoltification, proving that the changes observed in plasma concentrations of total T_4 or T_3 are associated with proportional changes in free, or diffusable, hormone levels.

Although most studies have reported that T_4 levels become elevated in smolting fish, hormone profiles and levels reported vary greatly. A long lasting (1–3 month) increase in plasma T_4 has been reported in coho salmon (Dickhoff et al. 1978, 1982), Atlantic salmon (Virtanen and Soivio 1985; Morin et al. 1989), and amago salmon, *O. rhodurus* (Nagahama et al. 1982), whereas a single shorter surge (1–2 weeks) has been reported in masu salmon (Yamauchi et al. 1985). Multiple surges have been reported in chinook salmon (Grau et al. 1982) and Atlantic salmon (Bœuf 1987), while in the masu salmon, Yamada et al. (1993) reported a long-lasting (2–3 months) elevation of T_4 associated with several distinct short (<1 month) peaks. Two distinct peaks were reported in Atlantic salmon by Bœuf et al. (1989). A final surge in plasma T_4 level has been reported by several groups in Pacific and Atlantic salmon, associated with the new moon nearest the spring equinox, and thought to induce downstream migration (Leatherland et al. 1992).

Several factors may explain the diversity of reported changes in plasma T_4 concentration. Long sampling intervals may not detect short peaks, as the one reported in masu salmon (Yamauchi et al. 1985). Lack of synchrony between the fish may mask changes in individual fish, since reported values are mean values of a group of fish and different fish are sampled each time. According to Bœuf (1987), stressful rearing conditions could induce such heterogeneity in physiological state and explain the occurrence of multiple surges, which were associated with low T_4 peaks and high interindividual variation in both chinook salmon (Grau et al. 1982) and Atlantic salmon (Bœuf 1987). Plasma T_4 may have increased synchronously in small groups of fish in response to a common triggering mechanism, such as the new moon, once these fish had reached a physiological "readiness." Differences in water quality during rearing have also been proposed to explain differences in magnitude, duration, and timing of the T_4 surge (Yamada et al. 1993). There may also be genetic differences. Bœuf and Le Bail (1990) showed that, reared under identical conditions, stocks of Atlantic salmon originating in long rivers exhibited a plasma T_4 increase one month earlier than those originating from short rivers. Such a difference could be related to the necessity of stocks from long rivers to start migrating earlier than stocks from short rivers, in order to reach the sea during optimal seawater tolerance. The migration-associated peak could therefore occur either before, simultaneously to, or after the smoltification-associated one. When these peaks are close, they may appear like one single long-lasting peak.

Plasma T_4 increased also in underyearling coho salmon during spring, but peak levels were significantly lower than in yearling coho salmon undergoing smoltification (Dickhoff et al. 1982). Youngson et al. (1986) similarly found consistently lower T_4 levels in Atlantic salmon parr than in smolts. Plasma T_4 may thus increase in response to photoperiodic cues at all ages, but a complete smoltification may be dependent upon a sufficient level being

reached. The level of plasma T_4, in turn, may depend on the thyroid responsiveness, which may depend on size, age, or exposure to changing environmental conditions.

4.1.2.2. Smoltification-related effects of thyroid hormones

Early experiments, reviewed by Dickhoff and Sullivan (1987), indicated that treatment of young salmonids with thyroid hormones was able to induce "smoltification," which at that time was defined as the acquisition of a silvery coloration. However, these fish were unable to survive in seawater, and were therefore later qualified as "pseudo-smolts." Subsequent studies have focused on identifying which of the changes associated with smoltification could be regulated by thyroid hormones. These studies have been extensively reviewed (e.g., Leatherland 1982; Barron 1986; Dickhoff and Sullivan 1987; Hoar 1988; Bœuf 1993) and key references may be readily located in these papers. Possible effects of thyroid hormones during smoltification include

- silvering due to guanine and hypoxanthine deposition in the scales and the skin;
- increased migration tendency;
- olfactory imprinting (see also Hasler and Scholz 1983);
- mobilization of lipid stores;
- increased food conversion efficiency (anabolic effect);
- increased protein synthesis by liver cells;
- increased oxidative metabolism;
- increased seawater tolerance;
- changes in visual pigments (see also Browman and Hawryshyn 1994);
- appearance of the adult forms of haemoglobin.

The thyroid hormones could also be involved in the tooth eruption occurring during smoltification of coho salmon (Gorbman et al. 1982), since thyroid deficiency in mammals is known to slow tooth eruption (West 1990). It is also probable that deposition of guanine crystals in the swim bladder occurs concomitantly with their deposition in the skin and scales. Since such deposition decreases the permeability of the swim bladder (Alexander 1993), thyroid hormones may participate in rendering smolts more buoyant.

Thyroid hormones may interact with other hormonal factors. The growth-promoting effect of thyroid hormones is inhibited by hypophysectomy and enhanced by growth hormone treatment (Donaldson et al. 1979; Leatherland 1982; Hoar 1988). Similarly, the stimulatory effect of thyroid hormones on guanine deposition and associated silvering is enhanced by growth hormone (Hoar 1988; Bœuf 1993). Growth hormone may act by increasing 5'-deiodinase activity, potentiating the effect of T_4. In Atlantic salmon, the peaks in plasma T_3 and growth hormone occurred simultaneously, whereas plasma T_4 increased as both growth hormone and plasma T_3 decreased, suggestive of decreased T_4 to T_3 conversion (Bœuf 1993). Thyroid hormones may in turn regulate growth hormone receptors or protein binding (McCormick 1995). This could explain why the success of coho salmon after transfer to seawater may depend on the fraction of the T_4 surge completed in freshwater prior to transfer to seawater (Folmar and Dickhoff 1981; Dickhoff et al. 1982).

The effect of thyroid hormones on smoltification-associated changes depends on the physiological state of the fish, the concentration of thyroid hormones, the duration of elevated levels, and possibly whether the increase in thyroid hormones is progressive or abrupt (Dickhoff and Sullivan 1987). This is consistent with the complexity of thyroid regulation depicted in section 4.1.1 and the interaction of thyroid hormones with other hormones. This also explains the difficulties encountered in defining the precise role of thyroid hormones during smoltification. According to Hoar (1988) "it is now clear that thyroid hormones do

not trigger the parr–smolt transformation. (...) The thyroid seems to play an important role in enhancing smolting characteristics that are regulated endogenously or by other hormonal factors." According to Dickhoff and Sullivan (1987), however, thyroid hormones may play a central role in coordinating the developmental rates of a variety of physiological systems during the parr–smolt transformation. Thus thyroid hormones could trigger parr–smolt transformation, but only when applied at the right time.

A possible example of the importance of the changes in thyroid hormone distribution on their effects may be illustrated by studies on imprinting. Hasler and Sholz (1983) have demonstrated that olfactory imprinting could be induced by TSH injections. Pre-smolt coho salmon receiving TSH and simultaneously exposed to synthetic chemical (either morpholine at 5.7×10^{-10}M or phenetyl alcohol 4.1×10^{-8}M) for 3 weeks, later demonstrated the ability to track their treatment odor upstream, whereas those receiving ACTH, saline or no injection did not. These field observations were confirmed by electrophysiological studies (Hasler and Scholz 1983). TSH treatment of rainbow trout has also been shown to facilitate learning and memorization. Pre-smolt fish trained to associate a red flash with food were able to learn faster and retain information for longer periods if they had been treated with TSH (see Hasler and Scholz 1983). It is important that some imprinting occurs prior to migration, so that smolts may segregate between their home tributary and confluents met during downstream migration. Increased T_4 levels and T_3:T_4 ratio in brain have been evidenced early in smoltification, before the plasma T_4 peak (Specker et al. 1992), and T_4 to rT_3 conversion in the brain has been shown to increase progressively during smoltification (Morin et al. 1993). Morin et al. (1989) studied olfactory learning and changes in thyroid physiology during smoltification of Atlantic salmon. The authors found histological indices of increased thyroid activity during two periods of smoltification, separated by 30 d. The first period was concomitant with enhanced long-term memory and olfactory learning, allowing for olfactory imprinting, while the second was concomitant with enhanced olfactory learning and peak gill Na^+K^+-ATPase activity. Thyroid hormone levels in plasma were increased only during the second period, which was interpreted as an indication of increased incorporation of thyroid hormones in the brain during the first period (Morin et al. 1989).

4.1.3. Possible involvement of thyroid hormones in river migration

4.1.3.1. Migration and increased thyroid activity

Studies in several migrating species of fish, including diadromous but also marine fish, indicate that the thyroid gland is hyperactive at the time of migration (see Baggerman 1960; Woodhead 1975). Thyroid gland uptake of ^{131}I was increased in coho and sockeye salmon smolts (Baggerman 1960) and the responsiveness of the thyroid gland was increased in Atlantic salmon smolts and adults during the period of migration (Youngson et al. 1986; Youngson and Mc Lay 1989). The pituitary–thyroid axis is thus ready for acute release of thyroid hormones into the circulation when proper stimulation occurs.

It is generally believed that onset of migration is associated with a surge in plasma T_4, which may be induced by the new moon (Grau et al. 1981; Grau 1982; Yamauchi et al. 1985), increased river discharge (Youngson et al. 1983; Youngson and Simpson 1984), changes in water temperature, quality or turbidity caused by rainfall, ice breakup, the spring turnover of water layers in lakes, or warm and sunny days (Yamauchi et al. 1985; Lindahl et al. 1983). Putative interactions between environmental stimuli are supported by the finding that plasma T_4 is better correlated with the moon cycle in salmonids exposed to changing flow or water quality than in those exposed to constant conditions (Nishioka et al. 1985).

The finding that plasma T_4 peaked earlier in smoltification in Atlantic salmon from short rivers than long ones (Bœuf and Le Bail 1990) supports its role in determining onset of migration, since such timing allows the fish to reach seawater at maximal level of seawater tolerance, before desmoltification occurs (cf. section 1.3.1).

However, some authors have suggested that a decrease in plasma thyroid hormones was necessary before onset of migration could occur (Birks et al. 1985). Exposure of hatchery-reared Atlantic salmon to increased water flow increased plasma T_4 and slowed downstream migration (Youngson et al. 1989) and downstream migrating hatchery-reared steelhead trout had lower plasma T_4 levels than nonmigrants (Ewing et al. 1984b). These studies are further discussed below.

Plasma T_4 levels are, nevertheless, most often increased in migrating fish. Present knowledge indicates that these high levels may be secondary to motor activity (Youngson and Simpson 1984; Virtanen and Soivio 1985; Virtanen and Forsman 1987; Whitesel 1992; Youngson and Webb 1993). Therefore, it is uncertain whether for example the new moon induces the T_4 peak, leading secondarily to downstream migration, or induces a premigratory restlessness, leading secondarily to increased T_4 levels (Leatherland et al. 1992).

During upstream migration, plasma T_4 tends to decrease from river entry to the spawning grounds as a result of gonad development (Leatherland et al. 1989), but still seems to correlate with swimming activity (Youngson and Webb 1993).

4.1.3.2. Migration-related effects of thyroid hormones

Behavioral changes

Treatment with thyroid hormones may induce restlessness, preference for open areas, downstream orientation, reduction in aggressive behavior, and reduced swimming activity in juvenile salmonids (Godin et al. 1974; Iwata et al. 1990; Iwata 1995). The observed changes all favor downstream migration of river-living and normally territorial fish, through facilitating social interaction and passive downstream displacement. Changes in salinity preference have also been demonstrated following treatment of juvenile Pacific salmon with TSH or antithyroid substances such as thiourea and thiouracil (Baggerman 1960; Baggerman 1963).

There are, however, conflicting results as to the stimulatory effect of thyroid hormones on downstream migration. In juvenile steelhead trout, treatment with T_4 decreased migration tendency whereas thiourea increased migration tendency as compared to saline-injected controls (Birks et al. 1985). Birks et al. (1985) suggested that the high levels of plasma T_4 during smoltification could allow the fish to maintain position in the stream and that migration was initiated as plasma T_4 decreased. Thiourea was as effective when injected together with T_4 as alone, suggesting an extrathyroidal action (Birks et al. 1985). However, since thiourea and propylthiouracil (PTU) are structurally and pharmacologically related compounds, thiourea could act by blocking deiodination of T_4 by the PTU-sensitive deiodinase, resulting in decreased effect of T_4 despite high levels. Increased plasma T_4 following T_4 injection or flow challenge similarly reduced downstream migration in Atlantic salmon smolts (Youngson et al. 1989).

Several factors may explain these conflicting results. Specker et al. (1992) recently demonstrated that during the smoltification-associated plasma T_4 peak, muscle content of both T_3 and T_4 decreased. It is therefore possible that normal changes in plasma T_4 may not have the same effect on motor activity as injection-induced changes. One could for example assume that a putative stimulatory effect of thyroid hormones on motor activity is normally blunted by decreased blood–muscle flux during the normal plasma T_4 peak, while injection

of T_4 at another time period is followed by high muscular T_4 content and a greater activation of swimming muscles. The experimental fish in which thyroid hormones were associated with decreased migration tendency were, moreover, fed *at libitum*, in contrast with wild migrating salmonids. Hepatic T_4 deiodination and the number of thyroid receptors are affected by nutritional state (Eales 1988), thus thyroid effects on migration could be modulated by the nutritional state of the fish. Such a switch could be a central mechanism in the nutrition-dependent motivation to migrate. Näslund (1990) described a population of landlocked Arctic char that actively migrate to very productive lakes upstream from the lake of residence in spring and return to their lake of residence in autumn. In this particular case, migration to the feeding grounds may bring about an increase in weight up to 450% over the summer and the lake of residence also allows for some growth, which suggests that the fish are still in a good nutritional state in spring. The relation between nutritional state, thyroid physiology, swimming ability, and migration certainly deserves further study.

Stimulation of the central nervous system

Morin et al. (1989) showed a significant correlation between plasma levels of T_4 and resting heart rate in Atlantic salmon smolts and suggested that thyroid hormones influenced heart rate by affecting the excitability of the nervous system of fish. As in higher vertebrates, thyroid hormones could increase the number of adrenergic receptors, present in the heart of salmonids. In addition, the thyroid hormones may have direct stimulatory effects on the central nervous system of fish, adapted to the challenge of migration. T_4 treatment of goldfish, *Carassius auratus*, increased both the rapidity of transmission from the retina to the optic tectum, the amplitude of the evoked potential, and the rapidity of depolarization, shortening the refractory period (Woodhead 1975). The effects of the pituitary–thyroid axis on the central nervous system is discussed in detail in Hasler and Scholz (1983).

Metabolism

High levels of thyroid hormones induce lipolysis and glycogenolysis in fish (Woodhead 1975), providing energy substrates necessary for migration. Oxidative metabolism in the liver of the teleost fish *Anabas testudineus* (Bloch) was stimulated by thyroid hormones (Peter and Oommen 1993) and there is some evidence that T_4 influences oxygen consumption in fish when internal or external factors are making increased demands on the organism (Woodhead 1975), such as during migration or smoltification. In *Anabas testudineus*, T_3 and T_4 treatment increased the activity of cytochrome oxidase and alpha-glycerophosphate dehydrogenase, which indicate increased oxidative activity (Peter and Oommen 1993). These effects persisted following suppression of insulin or adrenaline by alloxan or propanolol. However, an additional effect on succinate dehydrogenase, a marker enzyme of the Krebs cycle, then appeared, indicating a complex interaction between thyroid hormones, insulin, and adrenaline on substrate oxidation in fish (Peter and Oommen 1993).

4.1.4. Possible involvement of thyroid hormones during salinity changes

4.1.4.1. Changes in thyroid physiology following salinity changes

Some reports indicate that the thyroid gland of teleost fish responds to alterations in the ambient salinity, but the available data are scanty and sometimes contradictory (Leatherland 1985). In most cases of transfer of salmonids to seawater the plasma T_4 concentration is unaffected or decreases, while plasma T_3 may also decrease (reviewed by Bœuf and Le Bail 1990). Specker et al. (1984) demonstrated a 9–16% decrease in T_4 secretion 1 h following

acute transfer of coho salmon smolts to seawater. The decrease was greater after smoltification than in early smoltification. After the reverse transfer to freshwater, plasma T_3 level sometimes increases in rainbow trout and brown trout (Bœuf 1987).

Ambient salinity clearly affects the changes in thyroid physiology occurring during smoltification. Juveniles transferred to seawater as pre-smolts, before the T_4 rise, did not exhibit any smoltification-associated rise in T_4 level (Folmar et al. 1982; Specker and Kobuke 1987) and the thyroid response to TSH was blunted as compared to freshwater controls even 10 weeks following transfer (Specker and Kobuke 1987). When Atlantic salmon (Bœuf 1987) or coho salmon (Dickhoff et al. 1982) were transferred to seawater during the period of elevated plasma T_3 or T_4, these levels abruptly decreased and remained low. Such premature transfer may lead to stunting, which is characterized by a virtual cessation of growth accompanied by low plasma T_4 levels, and resulting eventually in death (Folmar et al. 1982). In contrast, coho smolts transferred to seawater later in smoltification, during the phase of decreasing plasma T_4, exhibited a greater response to TSH than their freshwater controls (Specker and Kobuke 1987). Seawater thus caused an activation of the thyroid system when fish were transferred at a proper time. The mechanisms involved in these saltwater-induced endocrinological alterations are unknown. The influence of hormones involved in osmoregulation, such as prolactin, would be interesting to investigate.

Premature transfer is not only accompanied by a depression of the thyroid axis; stunted fish are largely panhypoendocrine with regression of most endocrine glands and low circulating hormone levels (Folmar et al. 1982). However, plasma levels of growth hormone are seven-fold higher in stunts than in normal seawater post-smolts (Bolton et al. 1987; Björnsson et al. 1988), suggesting a defect in growth hormone receptor mechanism and (or) in mediating systems. This is consistent with the suggestion by McCormick (1995) that thyroid hormones are necessary for the action of growth hormone through regulation of receptors or binding proteins.

Finally, it should be kept in mind that one main aspect accompanying seawater transfer of wild salmonids is the increase in nutrient availability, which is known to alter thyroid physiology. Prolonged food deprivation in salmonids is associated with reduced plasma thyroid hormone concentrations, a lowered responsiveness to TSH challenge, a depressed *in vivo* hepatic T_4 mono-deiodination rate, and a decrease in hepatic T_3 binding (Leatherland and Cho 1985; Eales 1988). Thus, onset of feeding accompanying seawater transfer can be expected to induce, by itself, an intense activation of the thyroid axis in wild fish, an effect which is not provided for in many experimental designs. The interrelation between nutritional-, smoltification-, seawater-associated activations of the thyroid axis and catch-up growth appears as yet another interesting field for aquaculture research.

4.1.4.2. Effects of thyroid hormones on seawater adaptation

Feeding thyroid hormones to coho and Atlantic salmon improved their salinity tolerance, but this effect apparently depended on prior increases in body weight, which by itself increases seawater tolerance (McCormick 1995). In mammals, one of the most central roles of thyroid hormones is the increase in membrane Na^+K^+-ATPase activity, leading to increased standard metabolism and heat production (West 1990). Dickhoff et al. (1977) reported that gill Na^+K^+-ATPase activity increased in coho salmon given low doses of T_4, but not high ones, indicative of a biphasic effect of thyroid hormones in fish. Immersion of juvenile coho salmon in freshwater containing a low dose (0.01 $mg{\cdot}L^{-1}$) of T_4 similarly caused an increase of gill Na^+K^+-ATPase within 1 day but a decrease by 3–4 days of exposure (see

Dickhoff and Sullivan 1987). A high dose (1 mg·L^{-1}) of T_4 caused a decrease of gill Na^+K^+-ATPase within 1 day (see Dickhoff and Sullivan 1987).

Leatherland (1985) injected fasted rainbow trout with T_4 or thiourea for 3 weeks prior to acute seawater transfer and confirmed the existence of a "protective role" of thyroid hormones for seawater transfer. Recently, Lebel and Leloup (1992; Leloup and Lebel 1993) showed that T_3 was required for the development of hypoosmoregulatory mechanisms in brown trout and rainbow trout transferred to seawater. In underyearling amago salmon, T_4 and growth hormone in combination increased gill Na^+K^+-ATPase activity, whereas each hormone alone had no effect on the enzyme (Miwa and Inui 1985). T_4 alone did not increase seawater tolerance, whereas GH alone did. The two hormones had the greatest effect when administered simultaneously (Miwa and Inui 1985).

The effect of thyroid hormones on seawater adaptation seems highly dependent upon the rate, duration, dose, and time of administration (McCormick 1995). Even one of the most enthusiastic fish thyroid researchers recently stated that "it is still difficult to specify the exact role of thyroid hormones in osmoregulation and seawater adaptation in salmonids" (Bœuf 1993). The complexity of thyroid hormone kinetics, their biphasic action, their interaction with other hormones changing during salinity transfers, the effect of developmental stage, nutritional stage, stress, and time of the year makes the elucidation of their activity a great challenge and a number of "false" negative or positive results might indeed be expected.

The putative effects of thyroid hormones on the seawater adaptation of other physiological systems such as metabolism and digestion, cardio-respiratory system and acid–base regulation are apparently unexplored in fish.

4.2. Corticosteroids

The principal corticosteroids which have been isolated from blood or tissues of teleost fish are cortisol, cortisone, and corticosterone. Their relative concentrations may vary greatly within species or within the same individual at different phases of the life cycle (Woodhead 1975). In salmonids, plasma corticosterone concentration is usually low compared to plasma concentrations of cortisol or cortisone (Idler et al. 1964). Plasma cortisone may be slightly higher than plasma cortisol in undisturbed salmonids, but stress or seawater transfer results in long-lasting changes in the cortisone:cortisol ratio (Patino et al. 1987; Pottinger and Moran 1993). Aldosterone is secreted in minuscule amounts in salmonids, if at all (Patino et al. 1987). Wendelaar Bonga (1993) suggested that regulation of energy metabolism and osmoregulation are so closely linked in purely aquatic animals that these functions can be easily combined in one hormone.

4.2.1. General physiology of corticosteroids in fish

4.2.1.1. Hormone production

In teleosts, corticosterone and cortisol are produced in the interrenal cells, which are intermingled with catecholamine-producing chromaffin cells around the posterior cardinal veins in the head kidney (Wendelaar Bonga 1993). Cortisol is the major corticosteroid produced by the interrenal tissue of salmonid fish, while cortisone is formed by peripheral conversion of cortisol in various tissues, such as the spleen, the heart, and the gills (Patino et al. 1987). Although the steroidogenic mechanisms are basically the same as those of other vertebrates, fish may be the only vertebrates to 17β-hydroxylate 17-deoxycorticosteroids, transforming for example corticosterone into cortisol (Sandor 1979). Ablation of the interrenal

tissue has been possible in American eels, *Anguilla rostrata*, but apparently not in salmonids. The use of the steroid receptor antagonist RU-486 is therefore of particular interest for studying the effect of corticosteroid deprival in salmonids.

Reported base levels of plasma cortisol are usually lower than 10 $ngmL^{-1}$ in hatchery-reared salmonids (Arctic char: Jørgensen et al. 1993; Brunsvik 1993; coho salmon: Sumpter et al. 1986; rainbow trout: Sumpter et al. 1986; Pickering and Pottinger 1989; Pottinger et al. 1992a; Pottinger and Moran 1993; brown trout: Pickering and Pottinger 1989; Atlantic salmon: Olsen 1993). However, repeated samples on cannulated Atlantic salmon suggest that cortisol may be secreted episodically, as in mammals (Nichols and Weisbart 1984). In one study, base levels lower than 10 $ngmL^{-1}$ were interrupted by short-term variations (1–2 h) peaking at 60–80 $ngmL^{-1}$ (Nichols and Weisbart 1984), while in another study, an even greater plasma cortisol concentration range, 15–250 $ngmL^{-1}$, was found within or between resting individuals (Nichols and Weisbart 1985). These observations may explain the variability frequently observed in plasma cortisol concentration in salmonids. Under such conditions, large sampling groups are necessary to get a representative idea of mean plasma levels of cortisol. It is, however, difficult to prove that the peaks in plasma cortisol observed in cannulated Atlantic salmon were not associated with a stress reaction. The average plasma cortisol concentration which is reported in many salmonids is low, suggesting that large episodic peaks, such as those reported in cannulated Atlantic salmon, may not be a general feature of interrenal secretion in salmonids.

Fasting (Milne et al. 1979; Virtanen and Soivio 1985; Barton et al. 1988), sexual maturation (Audet and Claireaux 1992), sex (Mazeaud et al. 1977), size (Heath et al. 1993), and physical activity (Fagerlund 1967; Christiansen et al. 1991; Virtanen and Forsman 1987) may affect mean plasma cortisol concentration in salmonids. Daily and (or) seasonal variations in plasma cortisol concentration may occur (Spieler 1979; Audet and Claireaux 1992). Both photoperiod and feeding may regulate the daily rhythm of plasma cortisol concentration (Boujard et al. 1993; Spieler 1992). According to Nichols and Weisbart (1984), the nocturnal rise in mean plasma cortisol levels reported in some salmonids may be due to a higher frequency of episodic peaks during the night, rather than higher base levels.

ACTH is considered to be the major secretagogue regulating cortisol synthesis in fish, as it is in higher vertebrates (Wendelaar Bonga 1993). In mammals, acute stimulation with ACTH increases cholesterol release from local cytoplasmic stores and cholesterol uptake through low density lipoprotein receptors and accelerates side chain cleavage, the limiting step in steroid hormone biosynthesis (West 1990). A latency period of 5–10 min between ACTH injection and increased plasma cortisol concentration was found in Atlantic salmon reared at 10°C (Nichols and Weisbart 1984).

Chronic stimulation with ACTH in conjunction with growth factors induces hypertrophy and increased corticosteroidogenic capacity of adrenocortical cells in mammals (West 1990). In rainbow trout, prior *in vivo* treatment with mammalian ACTH significantly increased the *in vitro* responsiveness of interrenal tissue, isolated and exposed for ACTH the next day (Gupta et al. 1985). This observation indicates that ACTH has a rapid trophic effect on the interrenal of fish. The increase in sensitivity to ACTH and in steroidogenic capacity of interrenal cells during smoltification (Young 1986) could therefore be due to increased secretion of this hormone.

ACTH release is stimulated by corticotrophin-releasing factor (CRF), released from hypothalamic fibers, which in some fish also contain arginine vasotocin, a nonapeptide involved in salinity transfer and reproduction (Wendelaar Bonga 1993). Other peptides (β-lipotropin and an N-terminal peptide) derived from the precursor proopiomelanocortin

(POMC) may be released together with ACTH. Since ACTH cells are located between prolactin cells and their regulatory hypothalamic neurons (Nishioka et al. 1988), these substances may be implicated in modulation of prolactin cell regulation. As in mammals, chronically increased plasma cortisol levels in cortisol treated or infected salmonids, block the cortisol and ACTH response to stress, by exerting a negative feedback effect at both the hypothalamic and pituitary levels (Sumpter et al. 1986).

In addition to ACTH, PGE_1 is a potent stimulator of cortisol synthesis in rainbow trout *in vitro* (Gupta et al. 1985). Prolactin may increase PGE_1 synthesis in osmoregulatory organs of fish (Horseman and Meier 1978), thus possibly stimulating cortisol synthesis through a paracrine pathway from renal tubular epithelium to interrenal cells. Gonadotropin appears to be an extremely potent stimulator of cortisol synthesis in coho salmon (Schreck et al. 1989) and may play a main role in the high plasma cortisol levels observed in maturing salmonids (see below). Growth hormone, thyroxine, angiotensin II, arginin vasotocin, catecholamines, and α-MSH may also have corticotropic activities (Wendelaar Bonga 1993).

4.2.1.2. Response of the pituitary-interrenal axis to stress

Cortisol secretion is stimulated in response to many stressful stimuli. In Atlantic salmon, plasma concentration of ACTH increased within 2 min following onset of stress, reaching a maximal level within 5 min in some individuals, increasing more slowly in others (Nichols and Weisbart 1984). After 32 min of continuous stress, plasma ACTH concentration was 5–8 times higher than the base level, whereas plasma cortisol concentration was 20- to 50-fold higher than base level (Nichols and Weisbart 1984). In coho salmon reared at 10°C, plasma ACTH and cortisol were increased after 2 and 8 min of stress, respectively (Sumpter et al. 1986). A similar time relation was found in rainbow trout reared at the same temperature (Sumpter et al. 1986).

The magnitude of the increase in plasma cortisol concentration and the time course of the changes, appear to depend on the duration, application rate, and severity of the stressor (Barton et al. 1980). In most instances, however, plasma cortisol increases within 15 min and reaches or approaches maximal level within 1 h following onset of stress (Sumpter et al. 1986; Waring et al. 1992; Avella et al. 1991; Barton et al. 1980; Laidley and Leatherland 1988; Patino et al. 1987). The cortisone response has received less attention and seems to have a more variable pattern (Pottinger and Moran 1993). Interestingly, changes in plasma cortisone may occur much faster than changes in plasma cortisol (Pottinger and Moran 1993).

The cortisol response to stress varies in different species (Davis and Parker 1983), families (Heath et al. 1993), and individuals, "high- and low-responders" showing a consistency in their response (Pottinger et al. 1992b). Sex (Fagerlund 1967; Mazeaud et al. 1977), size (Barton et al. 1985), smoltification (Young 1986), and disease status (Sumpter et al. 1986) may also affect the cortisol response to stress, as well as water temperature and water quality (Pickering and Pottinger 1987).

4.2.1.3. Blood transport and metabolism

Once in plasma, cortisol binds to transport proteins. This binding protects it from metabolism and renal clearance (Nichols et al. 1985; West 1990). Corticosteroid protein binding systems in salmonids are similar to those in mammals, except that the association constant of the transcortin-like protein is lower and the binding capacity higher (Nichols et al. 1985). Therefore, plasma cortisol levels during episodic fluctuations, diurnal change, or after ACTH treatment do not normally exceed the binding capacity of the high-affinity

system in fish, and the proportion between bound and free cortisol is constant under such conditions (Nichols and Weisbart 1984). Following stress or seawater transfer, however, plasma cortisone increases (Patino et al. 1987; Pottinger and Moran 1993) and may compete with cortisol with respect to binding to plasma proteins (Nichols and Weisbart 1985).

A large proportion of corticosteroids are eliminated through the bile, where an accumulation of cortisol and its metabolites can be evidenced by 2 h after onset of chronic stress (Pottinger et al. 1992a). Determination of the bile content of corticosteroids has therefore been proposed as a useful means of detecting chronically elevated plasma cortisol levels when plasma cortisol may be acutely increased due to sampling stress, such as when sampling wild fish (Pottinger et al. 1992a). Such a method may be of particular value for studying the involvement of corticosteroids during migration.

4.2.1.4. Corticoid receptors

Glucocorticoids are lipophilic molecules that diffuse through the cell membrane and bind to intracellular receptors. While most steroid receptors are located predominantly in the cell nucleus, receptors for glucocorticoids are located in the cytoplasma and are taken up by the nucleus only when bound to their specific ligand (Parker 1995). This is consistent with the finding of Weisbart et al. (1987) of a positive correlation between cortisol concentration and cortisol receptor activity in gill nuclear extracts, whereas cytosolic cortisol concentration was positively correlated with plasma cortisol concentration but not with cytosolic receptor concentration. This indicates that glucocorticoids may not need any carrier molecules other than their specific receptors to cross the hydrophilic phase represented by the cytosol. Once in the nucleus, the ligand-receptor complex regulates gene expression (Alberts et al. 1994).

Glucocorticoid receptors appear to be down-regulated in response to elevated plasma cortisol in mammals and may be so in salmonids as well. Pottinger (1990) found a significant reduction in cortisol binding capacity of rainbow trout hepatocytes following 96 h confinement stress or chronic treatment with cortisol implants. Weisbart et al. (1987) similarly found decreased cytosolic cortisol binding capacity in gill tissue of brook trout following injection of cortisol or when plasma cortisol was elevated due to seawater transfer. In the latter study, nuclear binding capacity increased concomitantly, indicating that cortisol binding may have displaced receptors from cytosol to nucleus (Weisbart et al. 1987). In coho salmon, chronic stress or ingestion of a single meal containing cortisol induced a decrease in the number of gill receptors but an increase in the number of leukocyte receptors (Maule and Schreck 1991). Acute stress had no effect on gill receptors but increased the number of leukocyte receptors. Stress most often decreased the affinity of cortisol receptors. There is thus a tissue specificity in the downregulation of cortisol receptors (Maule and Schreck 1991).

In addition to their classical genomic action, steroid hormones can probably exert nonclassical action that is characterized by rapid effects of short duration occurring mainly at the membrane level. These effects include changes in membrane fluidity and regulation of, and (or) binding to, membrane receptors (Brann et al. 1995).

Finally, cortisol is converted to cortisone which may have a specific role in teleosts, for example in hepatic gluconeogenesis, ionoregulation, and regulation of cortisol entry into target tissues (for references, see Patino et al. 1987). Cortisone is believed to be far less active than cortisol, because it binds to plasma proteins about ten times more strongly than cortisol (Freeman and Idler 1973) and its binding to liver or leucocyte receptors is much lower than that of cortisol (Pottinger 1990; Maule and Schreck 1990). In isolated gill filaments of coho

salmon, cortisone was indeed less active than cortisol or dexametasone in increasing Na^+K^+-ATPase activity (McCormick and Bern 1989). However, no serious attempts have been made to identify specific cortisone-binding sites (Pottinger and Moran 1993). Regulation of the conversion of cortisol to cortisone could therefore regulate potential cortisone-mediated effects of cortisol. The different patterns of change in plasma cortisol and cortisone concentration during stress exposure of two strains of rainbow trout (Pottinger and Moran 1993), or during seawater exposure of coho salmon at two different periods (Patino et al. 1987), suggest that this conversion may be regulated in quite a complex way.

4.2.2. Possible involvement of corticosteroids in preparatory adaptations to salinity changes

4.2.2.1. Changes in corticosteroid physiology during and after smoltification

Interrenal cells undergo hypertrophy (see Specker 1982) and plasma concentration of cortisol increases during smoltification of coho salmon (Specker and Schreck 1982; Barton et al. 1985; Young et al. 1989) and Atlantic salmon (Langhorne and Simpson 1981; Virtanen and Soivio 1985). Peak titers of plasma cortisol often occur after maximal plasma thyroxine levels are achieved (Specker and Schreck 1982; Virtanen and Soivio 1985; Young 1986; Young et al. 1989). In coho salmon (Young et al. 1989), an initial rise to a plateau level maintained during 4–6 weeks, and coinciding with enhanced hypoosmoregulatory ability, was followed by an additional sharp rise to peak levels, coinciding with decreased hypoosmoregulatory capacity. Interestingly, this peak was also observed in seawater adapted smolts, suggesting that an endogenous seasonal rhythm of plasma cortisol exists in coho salmon, which is maintained irrespective of environment (Young et al. 1989). Such a rhythm would be particularly adapted to drive the cycle of migration observed in Norwegian Arctic char, migrating to the sea after the initial rise in plasma cortisol and returning to freshwater after the second rise, 3–8 weeks later (see Finstad et al. 1989; Finstad and Heggberget 1993; Berg and Berg 1993; Nilssen et al. 1997).

In concordance with the interrenal hypertrophy, Young (1986) reported a marked, significant increase in the *in vitro* interrenal sensitivity to ACTH and in its steroidogenic capacity during smoltification of coho salmon. Maximal responsiveness was concomitant with peak plasma thyroxine, enhanced hypoosmoregulatory capacity, and increasing plasma cortisol concentration. Young and Lin (1988) showed that increased responsiveness to ACTH could be induced by thyroid hormones. Peak plasma cortisol concentration occurred one month later and was concomitant with decreasing hypoosmoregulatory capacity (Young 1986). The cortisol response of coho salmon to acute stress similarly increased during smoltification (Barton et al. 1985). However, peak cortisol response to stress occurred at the time of peak cortisol base level. During the earlier plateau of moderate base levels, the stress response was moderate (Barton et al. 1985). One could therefore speculate that increasing responsiveness to ACTH, possibly induced by thyroid hormones, may cause interrenal hypertrophy and a moderate increase of plasma cortisol to a plateau level of about 20–25 $ng{\cdot}mL^{-1}$ in coho salmon (Barton et al. 1985; Young et al. 1989). Plasma cortisol may stabilize to a moderate plateau level rather than increasing further as the result of a progressive decrease in plasma cortisol half-life during smoltification (Shrimpton et al. 1994) or a decrease in interrenal sensitivity to ACTH. Four to six weeks later, some other changes, associated with lower interrenal sensitivity to ACTH (Young 1986) but with maximal plasma cortisol responsiveness to stress (Barton et al. 1985), could induce peak plasma cortisol levels, reaching 50–75 $ng{\cdot}mL^{-1}$ in coho salmon (Barton et al. 1985; Young et al. 1989;

Shrimpton et al. 1994). Environmental inputs at central level could be enhanced, causing hypothetically massive release of ACTH or other corticotropic hormones (cf. section 4.2.1.1).

Following the above-mentioned changes in cortisol dynamics associated with smoltification, coho smolts kept in freshwater may show a decrease in seawater tolerance. Shortly after, in July, a second rise in interrenal responsiveness to ACTH and stress may occur, concomitant with high plasma thyroxine (Barton et al. 1985; Young 1986). Young (1986) proposed that this second rise in interrenal sensitivity could be associated either with resmoltification or freshwater readaptation. When salmon are kept in freshwater, it seems that a resmoltification process may occur at that time (Young 1986), possibly related to the autumn downstream migration. When smolts are transferred to seawater in spring, the same hormonal changes in late summer may possibly induce migratory restlessness towards freshwater migration and energy mobilization towards a freshwater readapting process, which would fit the yearly migratory pattern of some salmonids. Different hormonal environments in freshwater and seawater, in particular different circulating levels of prolactin and growth hormone, may influence the direction of the changes. Cortisol could be a "transforming" hormone, inducing either smoltification or desmoltification depending on other endocrine factors. Such an ability has been shown for thyroxine, which can induce either disappearance or reappearance of ultraviolet photosensitivity in rainbow trout, depending on the stage of development (Browman and Hawryshyn 1994). This may be an important element of the plasticity of salmonids.

Corticosteroid receptor concentration and affinity decreased in the gills of smoltifying coho salmon, both in hatchery-reared and wild fish (Shrimpton et al. 1994). The changes were clearly correlated with development of seawater tolerance and smolt morphology, which led Shrimpton et al. (1994) to propose that low receptor affinity may be a useful parameter for evaluating smolting in coho salmon. The changes began to occur before plasma cortisol concentration increased, indicating that factors other than downregulation of receptors by cortisol must be involved. Such a regulation, mediated by a specific factor other than cortisol itself, easily allows a change in receptor density in some cortisol-receptive tissues but not all, as was shown in stressed coho salmon by Maule and Schreck (1991) (cf. section 4.2.1.4). In smoltifying coho salmon, the magnitude of the changes in gill corticosteroid receptor concentration and affinity were greater in wild fish, which also had the greatest concentration of receptors prior to migration and the best seawater tolerance as smolts (Shrimpton et al. 1994). Shrimpton et al. (1994) suggested that stressful rearing practices within the hatchery lead to a downregulation of corticosteroid receptors, decreasing the sensitivity of hatchery fish to the cortisol surge in the spring, thus possibly explaining their lower seawater tolerance.

4.2.2.2. Smoltification-related effects of corticosteroids

Development of hypoosmoregulatory capacity

High cortisol level during smoltification may increase the hypoosmoregulatory capacity of smolts by inducing proliferation and differentiation of chloride cells, as well as by stimulating intestinal fluid uptake. Cortisol treatment enhanced seawater tolerance of rainbow trout (Madsen 1990a), juvenile Atlantic salmon (Bisbal and Specker 1991; McCormick 1996), and desmoltified coho salmon (Richman and Zaugg 1987). Gill density of chloride cells increased as well as gill Na^+K^+-ATPase activity and residual (Na^+K^+-independent)-

ATPase activity (Richman and Zaugg 1987; Madsen 1990b; Bisbal and Specker 1991; McCormick 1996). Increased level of Na^+K^+-ATPase mRNA was shown in cortisol-treated brown trout (Madsen et al. 1995). Heavier osmium staining of chloride cells suggested that they were undergoing ultrastructural changes (Madsen 1990b). In isolated primary gill filaments from coho salmon, cortisol caused a significant dose-dependent increase in gill Na^+K^+-ATPase activity, indicating that cortisol may act directly on gill tissue (McCormick and Bern 1989).

Increased Na^+K^+-ATPase activity in the gills and enhanced seawater tolerance may, however, occur when plasma cortisol levels are low, as shown in coho salmon in autumn (Young et al. 1989) and in brook char during summer (Audet and Claireaux 1992). Moreover, cortisol treatment to yearling coho salmon in June, yielding a mean plasma cortisol concentration of 185 $ng{\cdot}mL^{-1}$, even decreased gill Na^+K^+-ATPase activity by 50%, although not affecting the seawater tolerance of the fish (Redding et al. 1984). Langdon et al. (1984) similarly found that 1–2 weeks of cortisol treatment decreased total Na^+K^+-ATPase activity in fully silvered Atlantic salmon smolts, while it had no effect in parr or pre-smolts. These results strengthen the hypothesis based upon the occurrence of peak cortisol levels during decreasing seawater tolerance (see above) that cortisol may be implicated in preadaptory structural changes either towards seawater adaptation or towards freshwater adaptation. Evidence for a role of cortisol in ion uptake are further discussed by McCormick (1995). The environmental or hormonal context may thus play a crucial role on cortisol-induced effects.

The increase in Na^+K^+-ATPase activity induced by cortisol treatment in Atlantic salmon was at the lower range of what is usually found in smoltifying fish, indicating that other hormones also participate in increasing gill Na^+K^+-ATPase activity during smoltification (Bisbal and Specker 1991). In Atlantic salmon, cortisol treatment seemed mainly to trigger the initiation of increasing gill Na^+K^+-ATPase activity, which continued to increase while plasma cortisol concentration decreased (Bisbal and Specker 1991).

In addition to its effect on gill structure and function, *in vivo* cortisol treatment enhanced intestinal fluid absorption and (or) intestinal Na^+K^+-ATPase activity in rainbow trout (Madsen 1990a) and Atlantic salmon (Cornell et al. 1994; Veillette et al. 1995). Veillette et al. (1995) showed that the steroid antagonist, RU 486, was able to inhibit the increase in intestinal fluid normally occurring during smoltification of Atlantic salmon. Since exogenous cortisol at physiological doses increased intestinal fluid in parr and post-smolts to the same rate as that of untreated smolts, Veillette et al. (1995) concluded that cortisol mediates the smoltification-associated increase in intestinal fluid absorption in Atlantic salmon. Cortisol did not have any immediate effect on intestinal water transport in the intestine *in vitro* (Usher et al. 1991a), suggesting that cortisol stimulates water transport through structural or other time consuming changes. Cortisol did not affect kidney content of Na^+K^+-ATPase mRNA in the brown trout (Madsen et al. 1995).

Increased seawater tolerance following cortisol treatment is typically apparent after about one week, supporting the notion that cortisol is a long-term adapting hormone, and plays an important preadaptory role for seawater transfer during smoltification (Bisbal and Specker 1991). The cortisol peak should thus occur some time prior to seawater entry. Specker (1982) proposed that the interval between peak thyroxine concentration and peak corticosteroid concentration may be related to the length of the river, thyroxine inducing migration, and cortisol inducing development of seawater tolerance. If so, one would expect that species undertaking long migrations to the sea have a thyroxine peak prior to the

cortisol peak, whereas those undertaking short migrations have a cortisol peak prior to the thyroxine peak. This remains to be tested.

Energy mobilization and preadaptation to stress

Cortisol stimulates energy mobilization from protein and lipid sources (Wendelaar Bonga 1993), which may be of importance to fuel the changes associated with the parr–smolt transformation. Plasma content of amino acid increased in cortisol-implanted rainbow trout (Andersen et al. 1991b). Lipid mobilization was stimulated by cortisol treatment in coho salmon parr, but not in smolts (Sheridan 1986). The apparent refractoriness of smolts to cortisol was, however, probably due to an already maximal lipid mobilization prior to hormone treatment (Sheridan 1986).

Cortisol also increases the number of β-adreno-receptors in hepatocytes and red blood cells. Consequently, the glycogenolytic response to catecholamines increases and liver cells are prepared for accelerated energy production during stress (Perry and Reid 1993). Catecholamines also regulate proton extrusion from red blood cells, which in turn regulates their intracellular pH (Perry and Reid 1993). During conditions of external acidosis and hypoxy, which may occur during seawater transfer (cf. section 2.1.2), the alkalization of the red blood cell interior is important to enhance both the affinity and the capacity of haemoglobin oxygen binding. Chronic (10 d) elevation of cortisol, *in vivo*, or short-term (24 h) elevation, *in vitro*, caused significant elevation of internalized β-adrenoreceptors in red blood cells of rainbow trout. Upon exposure of red blood cells to hypoxia, these additional receptors may be rapidly recruited to the cell surface where they become functionally coupled to adenylate cyclase, potentiating the adrenergic stimulation of proton extrusion (Perry and Reid 1993).

Morphological and behavioral effects

Cortisol or ACTH may be involved in the darkening of dorsal, pectoral, and caudal fins in Atlantic salmon smolts. These changes have been reported to occur at the same time as the rise in plasma cortisol concentration (Virtanen and Soivio 1985) and darkening of the dorsal surface and fins can be induced by ACTH injection (Langdon et al. 1984). More generally, stress or cortisol treatment induce darkening of the skin of salmonids (Iger et al. 1995).

Cortisol may be involved in the behavioral changes occurring during smoltification. At that period, many salmonids change from being territorial to adopting a schooling behavior; they stop defending their territory and leave it (Iwata 1995). This could be associated with a decrease in dominant behavior, in which cortisol may be involved. There seems to be a close relation between dominance and plasma cortisol in salmonids. In hatchery-reared fish, plasma cortisol levels are low in dominant fish and high in subordinates (Iwata 1995). When coho salmon parr are implanted with cortisol, elevating levels to within physiological realistic concentrations, these fish never become dominant and assume truly subordinate behaviors (C.B. Schreck, Oregon, personal communication). Atlantic salmon treated with cortisol or ACTH were also reported to become "less excitable and were more easily netted than the sham-injected controls after 5–7 days" (Langdon et al. 1984).

4.2.3. Possible involvement of corticosteroids in upstream migration

4.2.3.1. Interrenal activity during upstream migration

Hyperplasia of the interrenal tissue and very high concentrations of plasma corticosteroids have been reported in upstream migrating and mature Pacific salmon

(Robertson and Wexler 1959; Woodhead 1975). The range of cortisol values is wide, typically 10–1000 $ng{\cdot}mL^{-1}$, and plasma cortisol concentrations above 3000 $ng{\cdot}mL^{-1}$ may occur (Fagerlund 1967; Woodhead 1975; McBride et al. 1986). These high levels are sometimes associated with decreased plasma clearance, which may occur in sexually mature and spawned Pacific salmon shortly before their death (Idler and Truscott 1963). Higher levels of plasma cortisol, possibly due to a higher responsiveness of the interrenal tissue, have been evidenced in upstream migrating Arctic char of different sizes, as compared to downstream migrants (Høgåsen and Prunet 1997).

Plasma cortisol values are generally higher when fish are caught in turbulent waters or rapids, at the end of migration when the fish rely increasingly upon body protein for a source of energy, and when fish undertake long migrations concomitant with gonad development (Fagerlund 1967; Woodhead 1975). Females show generally higher plasma cortisol levels than males and a higher interrenal response to stress or ACTH (Woodhead 1975; McBride et al. 1986). Plasma cortisone concentration is often higher than that of cortisol (Woodhead 1975).

Plasma cortisol concentrations lower than 25 $ng{\cdot}mL^{-1}$ have been reported in mature pink salmon caught with a beach seine and sampled within 8 min (McBride et al. 1986) and adult sockeye salmon caught one at a time by divers (Fagerlund 1967). These values are of interest since they indicate that traveling through a foreign environment is not a sufficiently strong stressor (Wardle 1981) to maintain constantly high cortisol levels, at least not in adults. However, these cortisol concentrations were obtained from fish caught either at river entry or below falls. These are areas with deep water where the fish may remain for some time, waiting possibly for freshwater adaptive processes to develop or waiting for the appropriate daytime to cross the water falls (Smith 1985). In contrast, upstream migrants caught in the turbulent waters of rapids, or in shallow waters with a swift current, consistently showed high cortisol levels, even when sampled within 3 min (Fagerlund 1967). In such situations, motor activity is enhanced, the visibility is reduced, and the fish are more exposed to sudden stressors such as predators or sudden obstructions. Increased locomotor activity is obviously an integrated part of migration, and stress should probably be considered as such also. Hormone values obtained from unstressed fish may not be representative of the average physiological state of migrants. Large variations in plasma levels of cortisol probably occur during migration.

4.2.3.2. Putative effects of corticosteroids during upstream migration

The advantages of interrenal hyperactivity during upstream migration are several. First, the interrenal may provide hormones necessary for sexual maturation (McBride et al. 1986). The teleost interrenal cells are able to synthesize progesterone and to metabolize androgenic hormones and parallels have been drawn between the morphology of the interrenal tissue of sexually mature migratory salmon and the androgen producing *zona reticularis* of the mammalian adrenal gland (see McBride et al. 1986). Schreck et al. (1989) showed that both ACTH and gonadotropin induced the secretion of large quantities of androstenedione from the interrenal of coho salmon. Second, cortisol facilitates the mobilization of lipids (Sheridan 1988b) and proteins (Andersen et al. 1991b), which provide substrates for production of germ cells and energy necessary for migration. In mammals, glucocorticoids ensure high plasma glucose concentration despite fasting and builds up glycogen stores in the liver (West 1990). In salmonids, easily available glucose rapidly provides energy for anaerobic metabolism during migration through rapids or up waterfalls. The gluconeogenetic efficiency of glucocorticoids in upstream migrating salmonids is indicated by the high plasma

glucose levels during migration and high liver glycogen stores as adults reach the spawning grounds (cf. section 1.3.2).

The effect of cortisol on plasma glucose and liver glycogen seems to depend on the cortisol concentration and the nutritional status of the fish. Cortisol treatment in rainbow trout under certain circumstances may lead to decreased hepatic glycogen and only high doses may elicit hyperglycemia and stimulate gluconeogenesis under experimental conditions (Madsen 1990a; Vijayan and Leatherland 1989). Upstream migrants have a high interrenal capacity and high energy stores and upstream migration is accompanied by an intense mobilization of both fats and proteins (cf. section 1.1.3.2) and sometimes also by the production of lactate. High substrate availability and fatty acid content of plasma could explain the high efficiency of gluconeogenetic activity during upstream migration, leading to high plasma glucose and glycogen stores.

In contrast to other salmonids, Pacific salmon undergo a rigid sequence of events consisting of upstream migration, spawning, and death some days later (McBride et al. 1986). A relation between interrenal hyperplasia and post-spawning death has been suggested (Hane and Robertson 1959), but evidence for this view is still lacking. The carp, Atlantic salmon, and Arctic char, which may spawn repeatedly, may have as high corticosteroid concentration as Pacific salmon (Hane and Robertson 1959; Woodhead 1975; Høgåsen and Prunet 1997). Pacific salmon prevented from maturing by castration may live for several additional years (Robertson 1961). An interaction between gonadal and interrenal steroids could be implicated. Differences in receptor density between Pacific salmon and other species remain to be explored.

4.2.4. Possible involvement of corticosteroids in downstream migration

4.2.4.1. Interrenal activity during downstream migration

Interrenal activity of downstream migrating salmonids has apparently received less attention than that of upstream migrants. In Halselva, Northern Norway, mean plasma cortisol concentration in downstream migrating wild Arctic char was above 100 $ng{\cdot}mL^{-1}$ (Høgåsen and Prunet, unpublished data), which was consistent with previous findings in wild coho salmon (Shrimpton et al. 1994) and chinook salmon (Mazur and Iwama 1993) smolts caught similarly in a counting fence. Wild migrating Atlantic salmon smolts captured by fly-rod and sampled within 3 min following hooking had a mean plasma cortisol concentration of about 50 $ng{\cdot}mL^{-1}$ (McCormick and Björnsson 1994).

High levels of plasma cortisol in downstream migrating smolts could result from environmental stress, increased motor activity during downstream migration, smoltification (Fagerlund 1967; Wardle 1981; Specker 1982; Virtanen and Forsman 1987), or a combination of these factors. The interrenal capacity or responsiveness to stress or motor activity is higher in smolts than in parr (Barton et al. 1985; Virtanen and Forsman 1987). Plasma cortisol concentration in wild chinook smolts caught in a counting fence significantly decreased, from 120 $ng{\cdot}mL^{-1}$ at capture to about 20 $ng{\cdot}mL^{-1}$ after 10 days recovery in a hatchery (Mazur and Iwama 1993). In contrast, wild Atlantic salmon smolts caught as downstream migrants in early April and held in a restricted section of a natural stream, still had high serum cortisol levels in mid-April (184 $ng.mL^{-1}$) and mid-May (122 $ng.mL^{-1}$), compared to hatchery parr (<10 $ng{\cdot}mL^{-1}$) (Langhorne and Simpson 1981). Further experiments are needed to determine which of the different elements — migration, river environment, capture, or smoltification — are the major inducers of the high levels observed.

4.2.4.2. Putative effects of corticosteroids during downstream migration

Putative effects of corticosteroids include induction of migration, metabolism, protection against stress, olfactory learning, and preadaptation to seawater life.

High plasma cortisol level has been proposed to induce downstream migration by reducing the fish's awareness and ability to resist against the stream, due to osmoiono-regulatory disturbance. Thorpe (1987) found that at the time of maximal gill Na^+K^+-ATPase activity, mean plasma cortisol in Atlantic salmon smolts was low during the day (<5 ng·mL^{-1}) but peaked at night (about 50 ng·mL^{-1}). He proposed that high cortisol levels at night caused sufficient water and electrolyte disturbance to induce muscle fatigue and disorientation of smolts, resulting in onset of migration as the smolts would be carried down the stream. However, stimulation of ion extrusion by cortisol has been documented in salmonids only through structural and time-consuming changes (Foskett et al. 1983; Bisbal and Specker 1991; Usher et al. 1991a). A possible rapid action of cortisol should not be discounted, but lacks experimental support in salmonids (McCormick 1995). An interaction between the daily variations of cortisol with that of other hormones should also be investigated. The time relation between the daily rhythms of plasma prolactin and cortisol regulates migratory behavior in the white-throated sparrow, *Zonotrichia albicollis*, as well as the salinity preference of the gulf killifish, *Fundulus heteroclitus* (Meier and Fivizzani 1980). Daily variations in plasma cortisol, which vary according to the season or physiological state, have been evidenced in several salmonid species (Audet and Claireaux 1992; Thorpe et al. 1987; Rance et al. 1982; Pickering and Pottinger 1983). During smoltification of Atlantic salmon, plasma cortisol levels during the day decreased (from about 8 to 2 ng·mL^{-1}) whereas plasma cortisol levels at night increased significantly (from about 8 to 50 ng·mL^{-1}) (Thorpe et al. 1987). Rapid effects on ion transfer, dependent on the interaction of cortisol with other osmoregulatory hormones such as prolactin or growth hormone, should be investigated.

Migrating Atlantic salmon smolts had low lipid and liver glycogen stores in comparison with parr (Virtanen and Forsman 1987). Swimming induced a rapid depletion of liver glycogen stores in smolts, while parr seemed to rely more on lipid stores (Virtanen and Forsman 1987). Thus corticosteroids may be of particular importance during downstream migration to allow mobilization of protein stores and maintenance of high blood glucose through gluconeogenesis.

Migrating fish are probably more often exposed to stress than stationary ones since they travel through a foreign environment and have to face sudden obstructions or enemies without having any safe resting place (Wardle 1981). Recent observations in mammals suggest that endogenous cortisol is important in preventing stress-activated defense mechanisms from overshooting and damaging the organism (Muck and Naray-Fejes-Toth 1994). This could be of particular importance during repeated or long-lasting stress reactions. As mentioned earlier, cortisol has so far been accused of causing the death of upstream migrating Pacific salmon, although evidence is lacking. A putative protective effect in downstream migrants would therefore be interesting to investigate.

The hypothalamo-pituitary-interrenal axis may be involved in olfactory learning or recognition of odors during migration. Treatment with ACTH alone was unable to induce olfactory imprinting in pre-smolt coho salmon, whereas treatments with TSH or TSH + ACTH were (Hasler and Scholz 1983). However, cortisol injections alter olfactory discrimination in salmon and ACTH have been implicated in memory retention in birds and mammals (Hasler and Scholz 1983). In rats, receptors for gluco- and mineralo-corticoids display

a high concentration and distinct distribution in the hippocampus and circulating corticoids facilitate spatial learning (Oitzl and de Kloet 1992). There is evidence that salmon learn sequentially several cues on their way to the feeding grounds and will home to the site of release rather than to the native river system if no trace of that river is present at the site of release (Harden Jones 1968; Brannon 1982; Sutterlin et al. 1982; Hansen et al. 1993).

Cortisol is involved in development of hypoosmoregulatory capacity (McCormick 1995) and high levels during downstream migration may therefore participate in increasing the seawater tolerance of downstream migrating smolts. Zaugg et al. (1985) showed a dramatic increase in gill Na^+K^+-ATPase activity in chinook salmon smolts released in a river and captured 714 km downstream, mean levels in these fish being 2.5-fold as high as maximal Na^+K^+-ATPase activity reached in the freshwater controls retained at the hatchery and 1.9-fold as high as fish adapted to 28‰ seawater for 208 days. The role of cortisol in this improvement of seawater adaptability during migration deserves further investigation.

4.2.5. Possible involvement of corticosteroids during salinity changes

4.2.5.1. Changes in corticosteroid physiology following seawater transfer

Seawater transfer of freshwater adapted salmonids is usually followed by a transient rise in plasma cortisol concentration. In yearling coho salmon, plasma cortisol peaked at 220 $ng{\cdot}mL^{-1}$ by 1.5 h, then rapidly declined, reaching 50–70 $ng{\cdot}mL^{-1}$ by 24 h, then stabilized at smolt level, 25–50 $ng{\cdot}mL^{-1}$, for 1 week to more than 3 months, and finally, in some cases, decreased to freshwater levels by 2–4 weeks (Redding et al. 1984; Young et al. 1989; Avella et al. 1990; Shrimpton and Bernier 1994). The interrenal cells appeared more active in seawater smolts than in freshwater smolts (Nishioka et al. 1982). In brook trout, plasma cortisol concentration similarly increased above 100 $ng{\cdot}mL^{-1}$ following seawater transfer, but no decline was observed during at least 3 days (Weisbart et al. 1987). By 2 days following transfer, cortisol secretion rate, total cortisol pool size, and interpool transport rates were increased in brook trout, whereas plasma cortisol clearance was unaffected (Nichols et al. 1985). In cannulated post-spawned Atlantic salmon exposed to a shift from freshwater to seawater, plasma cortisol concentration increased from 75 to 130 $ng{\cdot}mL^{-1}$ by 2 h, then returned close to freshwater level by 6 h (Nichols and Weisbart 1985).

The differences in the interrenal response to seawater transfer may reflect a different need for cortisol stimulation of hypoosmoregulatory mechanisms following seawater transfer. Atlantic salmon and coho salmon both preadapt to seawater life in freshwater and the level of preadaptation seems to be greater in Atlantic salmon (Young et al. 1989). In brook trout, salinity is the major stimulant for the development of salt extrusion mechanisms (McCormick 1994). The seawater-induced increase in plasma cortisol may thus be involved in complete development of seawater adaptation in brook trout, major completion of seawater adaptation in coho salmon, and play little role in Atlantic salmon. However, putative differences in the cortisol to cortisone conversion and in the effect of cortisone could also explain the different patterns of plasma cortisol changes following seawater entry.

The effect of smoltification on the cortisol response of yearling coho salmon to seawater transfer has been investigated by Young et al. (1995). In parr and post-smolts, plasma cortisol following seawater transfer increased 6-fold by 24 h (60 $ng{\cdot}mL^{-1}$ vs. 10 $ng{\cdot}mL^{-1}$). During the smolt period (indicated by maximal gill Na^+K^+-ATPase activity, minimal changes in plasma osmolality following seawater transfer, and high plasma cortisol concentration), plasma cortisol 24 h after transfer increased about twofold (60 $ng{\cdot}mL^{-1}$ vs. 20–30 $ng{\cdot}mL^{-1}$), except for the mid-period when no increase was observed (see Fig. 2). This

specific period corresponded to a sharp decrease in condition factor and an increase in growth hormone concentration following seawater entry, which could indicate maximal smolt development. The change in plasma cortisol concentration following seawater transfer of coho salmon thus appears to depend more on smoltification than on hypoosmoregulatory capacity alone, as judged by high gill Na^+K^+-ATPase activity and low changes in plasma osmolality following seawater transfer, since these variables were stable while the cortisol response changed (see Fig. 2). The apparent absence of increase in plasma cortisol at the period when GH rose in response to seawater transfer (Young et al. 1995) suggests that long-lasting (≥24 h) increased plasma cortisol may be a "second choice solution" for seawater adaptation, growth hormone possibly being the first choice.

Patino et al. (1987) similarly showed that changes in plasma cortisol following seawater transfer of coho salmon were lower in smolts than in parr. Plasma cortisone concentration, in contrast, increased more in smolts, which led the authors to suggest that higher ambient temperature may have accelerated the conversion of cortisol to cortisone in smolts, as compared to parr. Such temperature effect does not, however, account for the observed similar increases in plasma cortisol concentration following seawater transfer prior to and after smoltification in the study by Young et al. (1995). Therefore, some specific effect of smoltification may exist as well.

Transfer by itself may elicit a stress response associated with increased plasma cortisol (Strange and Schreck 1978). However, cortisol levels following an acute handling stress usually return to base levels within 24 h in coho salmon (Avella et al. 1991; Patino et al. 1987) and other salmonids (Barton et al. 1980; Pickering and Pottinger 1987; Pickering et al. 1982; Pickering and Pottinger 1989). Coho salmon transferred between freshwater containers had lower plasma cortisol levels by 24 h than those transferred from freshwater to seawater (Young et al. 1995). Freshwater transfer of seawater adapted coho salmon did not cause any change in plasma cortisol (Avella et al. 1990).

Cortisol metabolic clearance rate increased and protein binding decreased following seawater transfer of cannulated Atlantic salmon (Nichols and Weisbart 1985). Nichols and Weisbart (1985) suggested that increased metabolic clearance rate was due to a lower binding of cortisol to plasma proteins, allowing elimination or metabolism of a greater free fraction of cortisol. Indeed, Nichols and Weisbart (1985) observed a decrease in plasma protein concentration, which probably resulted from extracellular volume expansion (cf. section 2.1.1.1). In addition, increased plasma cortisone concentration (Patino et al. 1987) may have displaced cortisol binding, since the affinity of cortisone for the common high-affinity binding sites are higher than that of cortisol (Nichols and Weisbart 1985). Changes in plasma pH accompanying salinity changes could also explain changes in binding to plasma proteins.

4.2.5.2. Effect of corticosteroids on development and maintenance of hypoosmoregulatory capacity

As mentioned earlier, cortisol may increase intestinal water absorption (Veillette et al. 1995) and induce proliferation and differentiation of gill chloride cells (Richman and Zaugg 1987; Madsen 1990b; McCormick and Bern 1989). These effects become apparent only after some days (Bisbal and Specker 1991; Usher et al. 1991a) to some weeks (McCormick 1996), reflecting the long-lasting, structural effects of cortisol. Following seawater transfer, the increase in cortisol is generally transient. In cortisol treated Atlantic salmon, however, gill Na^+K^+-ATPase activity kept on increasing despite decreasing levels of plasma cortisol (Bisbal and Specker 1991). Cortisol may thus have some triggering role and play a part in

seawater adaptation even if the increase is of short duration. Moreover, a rapid action of glucocorticoids on seawater adaptation could exist (McCormick 1995).

In seawater adapted teleosts, hypophysectomy, or interrenalectomy reduce branchial sodium efflux and gill Na^+K^+-ATPase activity and cortisol restores it towards normal (Foskett et al. 1983). In coho salmon with initially high gill Na^+K^+-ATPase activity, cortisol partially prevented the decline in activity normally occurring in isolated gills (McCormick and Bern 1989). Cortisol is therefore considered to play an important role in maintaining hypoosmoregulatory ability in seawater adapted fish. However, coho salmon stunts (Folmar et al. 1982) seem to be able to maintain normal levels of plasma sodium in seawater despite low corticotrophin cell activity and interrenal cell inactivity, as judged by electronmicroscopic examination (Nishioka et al. 1982).

Studies in tilapia opercular membrane indicate that other factors beside cortisol are involved in the complete differentiation of the functional chloride cell (Foskett et al. 1983). Although cortisol treatment induced proliferation and differentiation of chloride cells and increased Na^+K^+-ATPase activity, chloride secretion was not initiated. Injection of prolactin into seawater-adapted tilapia caused substantial inhibition of chloride secretion and conductance of the opercular membrane, suggesting that high levels of prolactin in freshwater may inhibit salt excretion. Kelley et al. (1990) demonstrated that cortisol decreased *in vitro* pituitary synthesis and release of prolactin. Therefore, cortisol could favor chloride secretion following seawater transfer by decreasing plasma prolactin. However, removal of prolactin by hypophysectomy followed by treatment with cortisol was not sufficient to induce salt excretion in tilapia (Foskett et al. 1983). Other factors, probably associated with the environment, are thus necessary to initiate salt excretion. Sodium chloride is efficient in inducing salt excretion, whereas osmolarity and calcium show no or little effect. Differentiation of chloride cells and chloride secretion probably requires a complex interaction between sodium chloride and hormones, especially cortisol (Foskett et al. 1983). Interestingly, a similar situation exists in the mammalian kidney. The cells of the connecting tubule are responsive to mineralocorticoids for the amplification of basolateral membrane, but the electrolyte composition of the diet seems to be decisive for stimulation of the transport processes in the cells (Kaissling and Kriz 1985).

4.2.5.3. Involvement of corticosteroids in freshwater adaptation

In his recent review on fish endocrinology, Wendelaar Bonga (1993) stated that corticosteroids should no longer be designed as seawater adaptive hormones, as they were equally important for development and maintenance of hyperosmoregulatory mechanisms in freshwater. This view fits with the putative involvement of corticosteroids in desmoltification, which was suggested by the patterns of variations of plasma cortisol (see above). However, experimental evidence for this view is limited. Laurent and Perry (1990) showed that cortisol treatment of freshwater-adapted rainbow trout resulted in increased whole body influx of sodium and chloride ions and increased number and individual apical surface area of gill chloride cells. Cortisol treatment may also increase gill H^+-ATPase, which is involved in sodium uptake (Lin and Randall 1993). However, seawater to freshwater transfer may not necessarily be associated with increased plasma cortisol (Avella et al. 1990). Further investigation in this field is required.

4.3. Prolactin

4.3.1. General physiology of prolactin in fish

Prolactin has an unusually wide spectrum of actions in vertebrates. It has been involved in regulation of water and salt balance, reproduction and lactation, growth and development, metabolism, behavior, immunoregulation, and structure of the ectoderm and skin (Clarke and Bern 1980; Loretz and Bern 1982; Hirano 1986; West 1990; Kelley et al. 1992; Gala 1991; Prystowsky and Clevenger 1994). Many of these effects are peculiar to certain species or groups, such as nest building, buccal egg incubation, and production of cropmilk (Hirano 1986). Speculating about the evolution of prolactin's functions, Nicoll (1980) underlined the versatility of prolactin: "Unlike the other hormones of the adenohypophysis, prolactin did not become specialized early in vertebrate evolution for the regulation of one or a few physiological processes. Instead, prolactin was used to control a wide variety of functions (...). This diversity of the hormone's actions suggests that it played an important role in the evolutionary diversification of vertebrates by being available for the regulation of the physiological functions of various emerging organs or processes." The diversity of action is also present in fish (Prunet and Auperin 1994) and particular care may be necessary for prolactin, as compared to other hormones, in generalizing from studies in one single species. Not surprisingly, this versatility and multiplicity of prolactin action is associated with structural variants of the hormone and its receptors (Sinha 1995; Prunet and Auperin 1994).

4.3.1.1. Structure of fish prolactin and consequences for prolactin research

Prolactin belongs to a family of structurally and functionally related polypeptide hormones that includes growth hormone, somatolactin, placental lactogen, and proliferin. It is a polypeptide of about 199 amino acid residues in tetrapods and slightly smaller, about 188 amino acids, in most fish, and its primary structure is known in representatives of all classes of vertebrates (Sinha 1995). Teleost prolactin was first isolated in 1983 from chum salmon by Kawauchi et al. (1983). Since then, it has been isolated from chinook salmon (Prunet and Houdebine 1984), Atlantic salmon (Andersen et al. 1989), rainbow trout (Mercier et al. 1989), and a number of nonsalmonid species (Sinha 1995).

In some fish, two variants of prolactin have been isolated (Prunet and Auperin 1994). In chum salmon and eel, the two forms are very similar and present strong homology, whereas those isolated from tilapia (prl_{188} and prl_{177}) are quite different and are thought to be expressed from distinct genes. Only in this last species has the biological significance of these two forms been studied and evidence indicates that they may have different osmoregulatory functions (Prunet and Auperin 1994).

Although it has long been known that fish prolactin differed functionally from tetrapod prolactins, therefore occasionally being named "paralactin" (Ball 1969a), most studies on the effects and plasma levels of prolactin in fish have been based on the more available ovine prolactin. The determination of prolactin gene sequences indicates that salmonid prolactin genes (as judged by chum, chinook, and rainbow trout prolactin) show 97–99% sequence homology among themselves, 67–73% homology with tilapia ($prolactin_{188}$), carp, and catfish prolactin genes, but only 29–35% homology with tetrapod prolactin genes (Sinha 1995). The amino acid sequences of fish prolactins show 60–80% homology among

themselves, but only 20–30% with mammalian prolactins (Prunet and Auperin 1994). Chum, chinook, and rainbow trout prolactins differ by only 1–6 amino acids (Mercier et al. 1989; Song et al. 1988).

Importantly, teleost prolactins lack one disulfide loop in the N-terminal region as compared with tetrapod prolactins, being thus more growth hormone-like (Sinha 1995). Chum salmon prolactin was 10–15 times more potent than ovine prolactin in stimulating the sodium-retaining activity of juvenile rainbow trout (Kawauchi et al. 1983). Reduction of the N-terminal bond of ovine prolactin increased the potency of the hormone 8- to 25-fold in a teleostean urinary bladder bioassay, suggesting that the N-terminal loop in tetrapod prolactin may hinder the binding of this hormone to a teleostean prolactin receptor (Doneen et al. 1979). Moreover, high doses of ovine prolactin may bind to growth hormone receptors. Indeed, ovine prolactin has been shown to bind similarly to prolactin and growth hormone receptors in tilapia liver (Prunet and Auperin 1994). In rainbow trout liver, however, growth hormone receptors were very specific to salmon growth hormone (Sakamoto and Hirano 1991). Binding of mammalian growth hormone to these receptors was 30-fold lower and binding of salmon prolactin was undetectable. Nevertheless, ovine prolactin showed some binding at high doses (Sakamoto and Hirano 1991). Hirano (1986) has reviewed the potency of different purified prolactins and growth hormones in several assay systems. Teleost growth is significantly enhanced by ovine prolactin, but less, or not at all, by fish prolactins (Hirano 1986). Russell and Nicoll (1990) have discussed the evolution of growth hormone and prolactin receptors and effectors.

It appears clear that early studies concerning the effect of prolactin on fish tissues should be considered with some criticism since either mammalian prolactin or unpurified fish pituitary extracts may have been used. Special attention should be given to results concerning metabolic effects of prolactin, since an interaction between ovine prolactin and growth hormone receptors has been evidenced in the liver. Moreover, there may be overlaps between the metabolic effects of prolactin and growth hormone. In contrast, the freshwater-adapting effect of prolactin shown in earlier studies is likely to be a prolactin effect, since only opposite, seawater-adapting effects of growth hormone have so far been shown. Characterization of prolactin receptors in fish with ovine prolactin is also considered as questionable (Nicoll 1975; Prunet et al. 1985; Prunet and Auperin 1994) and estimations of plasma prolactin concentrations in fish by the use of heterologous radioimmunoassay using tetrapod prolactin antigens and antibodies should always be validated by use of a homologous assay. Within salmonids, the different prolactins seem to behave very similarly in regard to binding to an antibody (Andersen et al. 1991a) or receptor (Prunet and Auperin 1994; Prunet et al. 1985). This was confirmed for the Arctic char by establishing a dilution curve with Arctic char pituitary extracts in a chinook-prolactin based radioimmunoassay (P. Prunet and H.R. Høgåsen, unpublished data).

4.3.1.2. Regulation of prolactin synthesis and release

Prolactin is synthesized mainly in the rostral pars distalis of the adenohypophysis. Extrapituitary sites of prolactin gene expression in mammals include the placenta and uterus, lymphocytes, and thymocytes (Sinha 1995), the mammary gland (Le Provost et al. 1994), and the brain (Emmanuelle et al. 1992; Dutt et al. 1994).

In most mammalian species, prolactin is synthesized as a prehormone consisting of 227 amino acid residues (Sinha 1995). Cleavage of the 28-amino acid signal peptide leads to the mature hormone, which is stocked within the pituitary and secreted in response to increased intracellular calcium and (or) cAMP (Nishioka et al. 1988). The recent identification of

several molecular forms of prolactin (glycosylated, phosphorylated, cleaved...) has led to the suggestion that prolactin perhaps is a prohormone, which is converted to different bioactive forms as it traverses the secretory pathway (Sinha 1995), a transformation which allows for a diversification of prolactin effects and of its regulation.

Diel rhythms of circulating prolactin are reported in a number of teleost species, including kokanee salmon (reviewed by Spieler 1979). However, these studies have to my knowledge not been confirmed with homologous assays. Using a salmon radioimmunoassay, no diel rhythm in plasma prolactin could be evidenced at three different dates in Atlantic salmon smolts sampled every 4 h over a period of 24 h (Prunet and Bœuf 1989). Cannulated rainbow trout similarly failed to show any diel rhythm in plasma prolactin (Prunet and Bœuf 1989).

Hypothalamic regulation of prolactin secretion

Unlike most other vertebrates, teleosts have a more or less direct neural control of pituitary function (Nishioka et al. 1988). A median eminence-portal system is generally considered to be absent from teleosts. Both peptidergic and aminergic hypothalamic fibers might control the prolactin cells. The released neurotransmitters must cross a basement membrane to enter the adenohypophysis, then a layer of ACTH cells surrounding the neurohypophysial processes, and finally stellate cells which are in direct contact with the prolactin cells. These cells may modulate movement of neurohormonal factors from the neurohypophysial nerve endings to the prolactin cells (Nishioka et al. 1988).

In most vertebrates, prolactin secretion is dominantly inhibited by hypothalamic factors. In many vertebrates, dopamine is the major prolactin release inhibiting factor (PIF). Teleosts may differ from this general pattern. Dopamine and more generally catecholamines seem to be absent from the pituitary of some fish species, suggesting that at least in these species, dopamine may not be a central PIF (Nishioka et al. 1988). Moreover, prolactin release from isolated rainbow trout pituitary cells decreases with time, suggesting that prolactin cells are under dominant stimulatory control by the hypothalamus in this species (Gonnet et al. 1989; Yada et al. 1991; Le Goff et al. 1992a).

Putative stimulatory hypothalamic substances for prolactin release in salmonids include thyrotropin releasing hormone and corticotropin releasing hormone (Nishioka et al. 1988). The latter has been suggested to increase prolactin during stress (Nishioka et al. 1988). However, the absence of correlation between the increases in plasma cortisol and prolactin during stress in salmonids (Avella et al. 1991; Pottinger et al. 1992c; Prunet et al. 1990) suggests that this putative effect may be mediated by a complex pathway. The complex interaction between prolactin and the hypothalamo-pituitary-interrenal axis in fish is discussed below. Recently, a putative specific hypothalamic prolactin-releasing peptide has been found in mammals (Hinuma et al. 1998). Its significance in fish remains to be studied.

Putative inhibitory hypothalamic substances for prolactin release in salmonids include dopamine, somatostatin, and γ-aminobutyric acid (GABA). These substances have all been identified in salmonid pituitaries (see Prunet et al. 1993; Le Goff et al. 1992a; Saligaut et al. 1990). Dopamine has been shown to inhibit prolactin release in several teleost species, including the rainbow trout (Nishioka et al. 1988). Somatostatin inhibited the release but not the synthesis of prolactin, in rainbow trout pituitary cells in primary culture (Le Goff et al. 1992a). Le Goff et al. (1992a) proposed that somatostatin acted as a modulator of hypothalamic stimulatory control. When tilapia was transferred from saltwater to freshwater, transport of somatostatin from the hypothalamus to the pituitary was inhibited, allowing for increased prolactin release (Nishioka et al. 1988). Transfer to seawater was accompanied by an apparent increase of the transport of somatostatin to the pituitary, possibly inhibiting

prolactin release. GABA has been shown to inhibit prolactin release by perifused pituitaries and isolated pituitary cells of rainbow trout (Prunet et al. 1993). Prunet et al. (1993) suggested that GABA may be responsible for an inhibitory tonus of prolactin cells, allowing for a rapid increase or decrease, within seconds, of prolactin release.

Extra-hypothalamic regulation of prolactin secretion

In several teleost species including the coho salmon, cytological studies *in vivo* as well as *in vitro* have correlated prolactin synthesis and release with environmental osmotic pressure (McKeown 1984). Prolactin cells are stimulated by hypoosmotic media and inhibited by hyperosmotic media. The estimation, with a homologous salmon radioimmunoassay, of the amount of prolactin released by isolated pituitary of rainbow trout, has confirmed the cytological findings (Gonnet et al. 1988). However, only large amplitude variations were efficient. Moreover, gonadotropin secretion followed a similar pattern as prolactin, which led Gonnet et al. (1988) to suggest that prolactin release could result from a nonspecific swelling of the endocrine cells. A similar conclusion was deduced from a study on hemipituitaries of coho salmon (Kelley et al. 1990). It is known from mammalian studies that cell swelling and resultant plasmalemma expansion is a potent inducer of prolactin and thryrotropin secretion from perifused adenohypophyseal cells (Wang et al. 1989). The mechanisms involved are unknown and may include stretch-activated calcium channels increasing intracytoplasmatic calcium concentration (Wang et al. 1989; Sato et al. 1990). Such a mechanism could be efficient in regulating prolactin release during the first days following the acute transfer of salmonids between freshwater and seawater, when a significant osmotic disturbance occurs (Gonnet et al. 1988).

Gonnet et al. (1988) also concluded that the rainbow trout prolactin cells were less sensitive to changes in osmolarity than nonsalmonid species studied so far. However, the sensitivity to osmolarity of salmonid prolactin cells may change during development. Cells from alevins, parr, and smolt are most often responsive, whereas those of mature fish appear less responsive (reviewed by Nishioka et al. 1988). Cell volume regulatory mechanisms could modulate the direct effect of osmolarity on prolactin cells. If the pituitary cells are able to regulate their volume as fast as the osmotic challenge, the prolactin response may be blunted. Na^+K^+-ATPase is known to play a crucial role in cell volume regulation (Alberts et al. 1994). Thus changes in the activity of this enzyme in pituitary cells during development should be investigated.

There appear to be differences in responses of salmonid prolactin cells to changes in osmolarity *in vivo* and *in vitro* (Nishioka et al. 1988). Nishioka et al. (1988) therefore suggested that some factor, possibly hypothalamic, could mediate the osmotic regulation of prolactin cells in intact fish.

Although increased prolactin secretion in freshwater was earlier ascribed to a direct effect of low environmental calcium, it is now clear that changes in external calcium levels do not affect plasma prolactin levels *in vivo* or *in vitro* provided in many cases that a minimal level is present (Nishioka et al. 1988; Sato et al. 1990; Arakawa et al. 1993; Prunet et al. 1990). The sodium content of the water may, however, be of importance. Transfer of rainbow trout to deionized water in which sodium was added did not result in any change in plasma prolactin, while transfer to artificial water deprived of sodium induced a decrease in plasma prolactin (Prunet et al. 1990). The significance of such a change is at present unknown (Prunet et al. 1990).

Prolactin cells of salmonids show seasonal variations in activity and prolactin release may depend on sexual status (Kelley et al. 1990; Hirano et al. 1985). An inhibitory effect of

melatonin on prolactin secretion has been proposed to explain the summer peak in plasma prolactin evidenced in Atlantic salmon (Andersen 1989). Treatment of immature rainbow trout with 17α-hydroxy-20β-dihydroprogesterone caused a significant decrease in plasma prolactin, whereas testosterone and estradiol-17β were ineffective (Prunet et al. 1990). Estradiol-17β was also ineffective in changing pituitary content of prolactin and prolactin mRNA in immature or ovariectomized rainbow trout (Le Goff et al. 1992b). Cortisol inhibited *in vitro* synthesis and release of prolactin by hemipituitaries of juvenile coho salmon (Kelley et al. 1990). Prolactin has been proposed to have an inhibitory effect on prolactin cells (Nishioka et al. 1988), but McKeown et al. (1987) could not find any binding of labeled homologous prolactin to the pituitary of rainbow trout.

4.3.1.3. Prolactin receptors

The prolactin receptor has recently been identified as a single-pass transmembrane protein, member of the hematopoietic/cytokine receptor superfamily, which is thought to be associated with a nonreceptor protein tyrosine kinase belonging to the Janus family (Prunet and Auperin 1994; Alberts et al. 1994). In mammals, varying length of the intracellular domain defines short-, intermediate-, or long-form classes, whereas in tilapia, one single class of prolactin receptors has been identified (Prunet and Auperin 1994). Using an autoradiography method, McKeown et al. (1987) have demonstrated the presence of specific prolactin binding sites in liver, intestine, kidney, bladder, skin, and gill of rainbow trout. Attempts to obtain specific binding of salmon prolactin to membrane preparations of salmonid tissues, as done in the tilapia, have so far been unsuccessful, which indicates that the concentrations of high-affinity receptor might be much lower in salmonids than in the tilapia (Prunet and Auperin 1994).

The majority of prolactin receptors in mammals has been located in intracellular membranes such as endosomes and Golgi and lysosomal structures, reflecting the rapid turnover of the receptors that, after synthesis and targeting to the plasma membrane, are rapidly internalized and degraded in lysosomes (Prunet and Auperin 1994). Such a rapid turnover of prolactin receptors allows for a fine and rapid regulation (Alberts et al. 1994). Cytoplasmatic prolactin receptors exhibiting a different affinity from that of the membrane receptors have also been identified in mammals, which may transport prolactin into the nucleus (Buckley et al. 1995). There, prolactin may bind to nuclear receptors immunologically identical to their membrane counterparts and induce actions such as proliferation of the cell (Buckley et al. 1995). The regulation of prolactin receptor density and turnover has not been investigated in salmonids. In mammals, prolactin receptors are affected by thyroid hormones, corticosteroids, gonadotropins, sex steroids, and prolactin itself. In tilapia, seawater transfer was accompanied by an increase of prolactin receptors in the gills (Prunet and Auperin 1994).

A recent study in mammals by Gertler et al. (1996) suggests that the activation of membrane prolactin receptors occurs through hormone-induced receptor dimerization, as earlier shown for growth hormone. Transient dimerization of the receptor lasting only a few seconds may be sufficient to initiate the biological signal. Interestingly, the interaction between homologous hormones and receptor was often of shorter duration than that of heterologous hormones (Gertler et al. 1996). This may explain different nonlinear dose-effect relationships, since high receptor occupancy may inhibit dimerization, thus further signal transduction. In certain human cell lines involved in immunological responses, prolactin stimulates DNA, RNA, and protein production only at low doses (Kelley et al. 1992).

4.3.1.4. Mediation and regulation of the effects of prolactin

The complexity of prolactin action is clearly indicated by the diurnal rhythm of several of its effects. Prolactin injections may either increase or decrease fat stores, depending on the time of the day when injections are made (Meier 1972). This has been documented in representatives of all classes of vertebrates, including fish. Daily variations in responses to prolactin have also been reported for the regulation of plasma chloride concentration and gonadal growth of killifish and antigonadal effects in killifish and sparrow (see Meier 1972; Meier and Fivizzani 1980).

In killifish, PGE_1 injections reproduced the hypochloremic effect of prolactin, whereas inhibitors of prostaglandin synthesis blocked the response to prolactin (Horseman and Meier 1978). Similar results were obtained in rat (Horrobin et al. 1978), suggesting that a prostaglandin-like substance, possibly PGE_1, could mediate some of the effects of prolactin in many species.

In killifish, the daily rhythm of fattening response to prolactin can be phased or entrained by thyroxine or cortisol treatment. Cortisol treatment consistently entrained the rhythm of fattening response in other vertebrate groups (see Meier 1972). Meier and Fivizanni (1980) therefore suggested that the temporal relation between the daily rhythms of plasma prolactin and cortisol could regulate the effects of prolactin. One mechanism could be that high levels of cortisol inhibit prolactin action by inhibiting prostaglandin synthesis. The simultaneous occurrence of high prolactin level and high cortisol level, could therefore have opposite effects as compared to the simultaneous occurrence of high prolactin level and low cortisol level (Meier and Fivizzani 1980). Because some effects of prolactin and PGE_1 in rats occurred only in males and only during winter (Horrobin et al. 1978), the existence of other regulatory factors, such as melatonin or sex steroids, could exist as well.

There is thus some possibility of a very close interaction of the hypothalamo-interrenal axis and prolactin activity in fish. Corticotropin releasing factor could stimulate prolactin synthesis or release, ACTH cells could interfere with the hypothalamic control of prolactin cells (Nishioka et al. 1988) and cortisol could inhibit prolactin-induced prostaglandin synthesis.

Finally, Nicoll et al. (1990) have provided evidence that in many groups of vertebrates, including teleosts, prolactin induces the synthesis by the liver of "synlactin," which synergizes prolactin action. By acting specifically on certain tissues and being specifically regulated, such substance could participate in increasing the complexity of action and regulation of prolactin.

4.3.2. Possible involvement of prolactin in preparatory adaptations to salinity changes

4.3.2.1. Changes in plasma prolactin during smoltification

Both histological observations and investigations of plasma prolactin concentrations suggest a role for prolactin in smoltification (Nishioka et al. 1982; Prunet et al. 1989). A progressive increase in plasma prolactin in February–March was reported in smoltifying coho salmon, followed by a sharp decrease in April, when hypoosmoregulatory ability was maximal (Young et al. 1989). In contrast, Richman et al. (1987) found that prolactin peaked at a time of high Na^+K^+-ATPase activity in the gills and elevated plasma thyroxine, in the same species. In Atlantic salmon smolts, plasma prolactin levels decreased in March and remained low until June, while plasma thyroxine and gill Na^+K^+-ATPase activity peaked in

April (Prunet et al. 1989). In another experiment, Atlantic salmon smolts showed ample variations in plasma prolactin during smoltification, stabilizing at low levels only in May, while parr showed a steady increase in plasma prolactin from February to June (Prunet and Bœuf 1989). In Atlantic salmon smolts kept in freshwater, prolactin concentration reached a level similar to that of seawater transferred smolts (Prunet et al. 1989). In coho salmon smolts, in contrast, prolactin level in freshwater always remained higher than following seawater transfer (Young et al. 1989).

Decreased plasma levels of hormones may be associated with increased flux of hormone to specific tissues. Such effects during smoltification are now well documented for thyroid hormones (cf. section 4.1). As described above, the receptor system of prolactin is a very dynamic one and one might expect that similar changes in tissue flow may occur during smoltification. This has, nevertheless, not been documented so far. On the contrary, Kelley et al. (1990) found a temporary decrease in prolactin synthesis from perifused pituitaries during parr–smolt transformation of coho salmon which correlated well with the decrease in plasma prolactin found by Young et al. (1989).

4.3.2.2. Effects of prolactin on smoltification

The precise role of prolactin during smoltification of salmonids is largely unknown. Barron (1986) suggested that high prolactin levels may be important in maintaining osmoregulatory homeostasis in the smolted salmonid before entering sea water, whereas Young et al. (1989) suggested that a decrease in prolactin may be necessary for Na^+K^+ATPase-activity to increase during smoltification.

An approach to understanding the role of prolactin in smoltification has been provided by Madsen and Bern (1992), who injected ovine prolactin and (or) ovine growth hormone into freshwater-adapted rainbow trout and brown trout and studied the effects of the different treatments on subsequent seawater adaptation. Prolactin treatment alone had no effect, but when injected simultaneously with growth hormone, it significantly abolished the seawater adaptive effect of growth hormone in a dose-related manner. The authors therefore suggested that prolactin is antagonistic to growth hormone during development of hypoosmoregulatory mechanism in salmonids, supporting the hypothesis of Young et al. (1989) (see above). However, implants of ovine prolactin did not significantly antagonize the effect of growth hormone treatment in presmolts (Bœuf et al. 1994). These implants even caused an increase in gill Na^+K^+-ATPase activity, although they did not improve seawater tolerance (Bœuf et al. 1994). In Atlantic salmon smolts showing high gill Na^+K^+-ATPase activity, cholesterol implants of ovine prolactin during two weeks did not impair seawater adaptability (Prunet et al. 1990). Homologous hormones are probably necessary to further study these aspects.

Prolactin is an important regulator of programmed cell death and cell proliferation during tissue restructuring, as shown during amphibian metamorphosis (Tata 1994). Such effects should be investigated during smoltification. Finally, a lipolytic effect of prolactin during smoltification remains to be investigated.

4.3.2.3. Prolactin and preadaptation to freshwater transfer

McKeown (1984) and Andersen et al. (1991a) have suggested that prolactin might be involved in preparing upstream migratory adult salmon to adapt to freshwater conditions. In support of this view, plasma prolactin increased in adult Atlantic salmon kept in seawater at the time of upstream migration (Andersen et al. 1991a). Interestingly, the prolactin peak was similar in maturing and immature fish, whereas only maturing Atlantic salmon migrate into

freshwater. The plasma concentration of sex steroids in maturing fish was higher than in immature fish (Andersen et al. 1991a), suggesting that sex steroids may modulate the effect of the prolactin surge. Such interaction could explain why males and females often leave the ocean at different times (Dempson and Kristofferson 1987).

The close interaction between prolactin and steroids on osmoregulatory, reproductive, and metabolic effects (Hirano et al. 1985; Hirano 1986) suggests the possibility of a complex interaction between these hormones. One may speculate that several variants exist in salmonids. Sex steroids may have a major role on prolactin effects in seawater-adapted Atlantic and Pacific salmon, which only return to freshwater to spawn, but only a minor effect in the Arctic char, which return to freshwater every year independently of sexual maturation. The modulation of the interaction between sex steroids and prolactin during evolution could be one element explaining the increasing degree of anadromy of salmonids, from *Salvelinus* to *Oncorhynchus* species (cf. section 3.2.2).

The increased plasma prolactin in seawater-adapted Atlantic salmon remains, however, a single example, which should be verified in other species before any generalization is drawn. In support of such a view, however, prolactin cells of sockeye and chum salmon have been shown to become active in fish prior to the entry into freshwater (McKeown 1984). High levels of prolactin in plasma and pituitary have been reported in chinook salmon still far from the mouth of the river. In contrast, no changes in plasma prolactin were observed from June to January in maturing female rainbow trout (Prunet et al. 1990). Maturing chum salmon showed a significant increase in plasma prolactin only once in the river, adults caught in the bay showing not even a tendency of increased plasma prolactin levels as compared to seawater level (Kakizawa et al. 1995). Differences in timing or difficulties in detecting short peaks could, nevertheless, explain these negative results.

4.3.3. Possible involvement of prolactin in river migration

There are at present few studies with salmonids that indicate that prolactin is implicated in migration. One may speculate from the above-mentioned studies, that prolactin may play some role in inducing the return migration of salmonids. This may also be the case for the three-spined stickleback, *Gasterosteus aculeatus* (see McKeown 1984). This teleost migrates from seawater to freshwater to spawn and increased activity of the prolactin cells during the spawning migration has been recorded. Prolactin is necessary in this species for osmoregulation in freshwater, but prolactin cells become activated long before the fish enter freshwater (see McKeown 1984).

Similarly, one may speculate that low levels of prolactin in smolts may favor osmoregulatory disturbance in freshwater, possibly inducing seawater preference and downstream migration. In Arctic char, plasma prolactin concentration was lower in downstream migrants than in stationary fish sampled during the period of downstream migration (Høgåsen and Prunet 1997). In Atlantic salmon smolts, Thorpe (1987) demonstrated maximal levels of plasma cortisol at night at the time of maximal gill Na^+K^+-ATPase activity and suggested that such levels might cause sufficient water and electrolyte disturbance to induce muscle fatigue and disorientation of smolts, resulting in onset of migration as the smolts would be carried down the stream. In view of the complex interaction between prolactin and the hypothalamo-pituitary-interrenal axis, including a putative inhibition of prolactin action by cortisol, it is indeed possible that the association between low prolactin and high cortisol, could induce onset of migration. The time relation between the daily rhythms of plasma prolactin and cortisol has been shown to regulate the salinity preference of the gulf killifish

and the migratory behavior of the white-throated sparrow, *Zonotrichia albicolli* (Meier and Fivizzani 1980).

The return of some amphibians, such a the newt, *Notophthalmus viridiscens*, to an aquatic environment, or "water drive," has been demonstrated to result from the action of prolactin (McKeown 1984). More work is needed before one can draw any conclusion on the effect of prolactin on migration in salmonids.

4.3.4. Possible involvement of prolactin during salinity changes

4.3.4.1. Changes in plasma prolactin following salinity transfer

Seawater transfer

A significant and long-lasting decrease in plasma level of prolactin has been reported following seawater transfer of rainbow trout, coho salmon, and amago salmon (Prunet et al. 1985; Prunet and Bœuf 1985; Young et al. 1989; Yada et al. 1992). In all cases, levels decreased within 24 h following transfer and remained low until the end of the experiment, i.e., from 1 week to 3 months later. The decrease was evident already by 7 h after transfer in rainbow trout (Prunet et al. 1985).

In contrast, Atlantic salmon smolts and chum salmon failed to show any decrease in plasma prolactin following seawater transfer (Prunet and Bœuf 1985; Hasegawa et al. 1987; Prunet et al. 1989). A short increase in plasma prolactin even occurred in Atlantic salmon on day one, which was attributed to a stress response since a freshwater to freshwater transfer also elicited an increase in plasma prolactin concentration (Prunet et al. 1989).

The variation in prolactin response to seawater transfer in different salmonid species could be directly related to their different level of preadaptation (Prunet et al. 1989). Rainbow trout and coho salmon do not preadapt to seawater life to the same extent as Atlantic and chum salmon do (see Prunet et al. 1989; Baggerman 1960). Rainbow trout, coho salmon, and amago salmon may therefore need a further decrease in prolactin to adapt to seawater. This decrease could be induced by cortisol (Kelley et al. 1990). Such an effect could explain why cortisol appears to play a central role in seawater adaptation of coho salmon, whereas work on the Atlantic salmon has so far failed to implicate cortisol in seawater adaptation (see Prunet et al. 1989).

The decrease in plasma prolactin following seawater transfer results both from decreased secretion and decreased synthesis of prolactin. In rainbow trout, the pituitary content of prolactin sharply increased by day 2 following seawater transfer, then progressively decreased, reaching freshwater level 3 weeks after transfer (Prunet et al. 1985). The short-term elevation of cortisol following seawater transfer (Redding et al. 1984; Young et al. 1989) could explain the immediate inhibition of prolactin release. While plasma cortisol and pituitary prolactin progressively returned to pre-transfer levels, plasma prolactin remained low, suggesting that another, long-lasting mechanism may relay the putative acute inhibition of prolactin release by cortisol. Numerous histological studies have shown that prolactin cells appear more active in freshwater than in saltwater (see Gonnet et al. 1988). The pituitary content of prolactin, prolactin synthesis rate, and prolactin release *in vitro*, were significantly lower in coho salmon smolts one month after seawater transfer than in freshwater controls (Kelley et al. 1990). However, the level of prolactin mRNA in the pituitary of amago salmon did not change significantly during the first week following seawater transfer (Yada et al. 1992), indicating that the decrease in prolactin synthesis may be delayed. The pituitary content of prolactin following long-term adaptation to seawater was higher or

similar to the pituitary content of prolactin in freshwater, in rainbow trout (Prunet et al. 1985), but lower in seawater than in freshwater, in coho salmon (Kelley et al. 1990).

Direct transfer of tilapia from fresh water to brackish water was accompanied, within 24 h, by a 2- to 7-fold increase in binding capacity of gill prolactin receptors (Prunet and Auperin 1994). These levels were maintained for at least 28 days, when the experiment ended. Prunet and Auperin (1994) suggested that the high binding capacity of gill membranes to prolactin in hyperosmotic medium could function as a preadaptation to freshwater transfer, allowing for a rapid effect of prolactin once the fish entered freshwater. The regulation of prolactin receptors in salmonid tissues has not been investigated.

Freshwater transfer

Plasma prolactin concentration has been reported to increase after freshwater transfer of seawater-adapted juvenile rainbow trout, juvenile chum salmon, adult Atlantic salmon, and mature chum salmon (Hirano et al. 1985; Prunet et al. 1985; Potts et al. 1989; Ogasawara et al. 1989). Changes in plasma prolactin were evident by 8 h in adult Atlantic salmon (Potts et al. 1989) but only by 2 days in juvenile chum salmon (Ogasawara et al. 1989). In the last mentioned study, prolactin peaked by 5 days and returned to pre-transfer level by 7 days. In mature chum salmon, plasma prolactin returned to seawater levels by day 2 in males and by 1 week in females (Hirano et al. 1985). In rainbow trout, plasma prolactin concentration increased within one day, reached a plateau level after 4 days, and stayed at this high level for at least 3 weeks (Prunet et al. 1985). In the last mentioned study, the pituitary content of prolactin decreased significantly within 2 days and remained low for at least 3 weeks, indicating that both synthesis and release were enhanced (Prunet et al. 1985).

The prolactin response to freshwater transfer may thus depend on species, sex, or physiological state. The physiological significance of the temporary increase in plasma prolactin following freshwater transfer of some salmonids remains unclear. It could be related to osmoregulation, reproduction, or stress (Prunet et al. 1990). Further investigations are required to clarify this effect.

Potts et al. (1989) observed the activation of hyperosmoregulatory mechanisms necessary for freshwater life in adult Atlantic salmon immediately following freshwater entry, whereas no change in prolactin levels was detectable for at least 8 h following the transfer. In other studies, the increase in plasma prolactin may require even longer. Indication of an activation of the internalization of growth hormone/receptor complexes in the liver has been provided following seawater transfer of rainbow trout (Sakamoto and Hirano 1991). A similar mechanism for prolactin following freshwater transfer, could explain the rapid effects of prolactin, despite little change in plasma concentration of prolactin.

4.3.4.2. Osmoregulatory effects of prolactin

Since the pioneer finding by Pickford and Phillips (1959) that hypophysectomized killifish required prolactin for survival in freshwater, prolactin has been recognized as the principal freshwater adapting hormone in teleosts. Numerous studies have confirmed the osmoregulatory effects of this hormone in fish, which in turn have brought about the gradual recognition of osmoregulatory roles of prolactin in other vertebrate groups (reviews: Ball 1969a, 1969b; Nicoll 1980; Loretz and Bern 1982; Hirano 1986; Prunet et al. 1990). It has been proposed that osmoregulatory actions and growth and (or) development effects may have been the "fundamental" functions of the hormone (Nicoll 1980). Prolactin has been shown to regulate ion and solute transport by acting on cell differentiation, cell polarization, cell proliferation, cell junctions, and activity of membrane transport systems

(Olivereau and Olivereau 1977; Cowie et al. 1980; Loretz and Bern 1982; Foskett et al. 1983; Flint and Gardner 1994; Sjaastad et al. 1993). The response to prolactin has been shown to require from only a few minutes to up to several days (Loretz and Bern 1982; Shennan 1994).

The effects of prolactin on osmoregulation in teleosts have been extensively reviewed (see e.g., Loretz and Bern 1982; Foskett et al. 1983; Hirano 1986; McKeown 1984). The major function of prolactin is probably to maintain a low permeability to water and ions in epithelia of the skin, the gills, the intestine, the renal tubules, and the bladder (Wendelaar Bonga 1993). In salmonids, the precise role of prolactin in osmoregulation is, however, unclear (Prunet et al. 1990). In contrast with the killifish, hypophysectomized salmonids such as the rainbow trout, Atlantic salmon, and coho salmon, survive in freshwater, showing only a slight decrease of plasma ion concentrations (Yamauchi et al. 1991). Still in contrast with the killifish, the same hypophysectomized fish do not survive in seawater (Yamauchi et al. 1991). Interestingly, in one study, Oduleye (1976) found that hypophysectomized brown trout (n = 20) survived in 33% seawater but not in freshwater, suggesting that prolactin may be necessary for freshwater survival in some salmonids. In this regard, it is interesting to note that both the Atlantic salmon and coho salmon are able to remain in seawater during the winter, whereas the brown trout usually spends only the summer at sea (L'Abée-Lund et al. 1989). One may speculate that there may be a difference in prolactin action in salmonids remaining at sea during winter and those returning to freshwater.

Autoradiography studies in the rainbow trout with radiolabelled homologous prolactin have shown that prolactin binds to liver, intestine, kidney, bladder, skin, and gill (McKeown et al. 1987), suggesting a role for prolactin in osmoregulation and possibly metabolism. Treatment of Atlantic salmon smolts and rainbow trout with ovine prolactin did not affect gill Na^+K^+-ATPase activity (Prunet et al. 1990). Recently, Madsen et al. (1995) showed that treatment of brown trout with salmon prolactin had no effect on the expression of gill and kidney Na^+K^+-ATPase. More studies on the osmoregulatory role of prolactin in salmonids is needed before any conclusion can be drawn (Prunet et al. 1990).

4.4. Other hormones

In addition to thyroxine, corticosteroids, and prolactin, other hormones are undoubtly involved in migration of anadromous salmonids. The hormones that have received some attention in the field of salmonid endocrinology for their role in migration or smoltification are discussed in the present chapter.

4.4.1. Growth hormone

4.4.1.1. General aspects

Growth hormone (GH) is a polypeptide that is structurally and probably evolutionary related to prolactin (Kawauchi et al. 1990). GH has been isolated and sequenced in several teleost species (Takei 1993). Two structural variants have been found in chum (Kawauchi et al. 1986) and chinook (Le Bail et al. 1989) salmon. Like prolactin, salmon GH shows only moderate amino acid sequence identity with mammalian GH (Kawauchi et al. 1990). Recombinant trout GH was at least 10 times more efficient than ovine GH for increasing gill Na^+K^+-ATPase activity and salinity tolerance in pre-smolts of Atlantic salmon (Bœuf et al. 1994). Regulation of GH synthesis and release in fish is reviewed by Nishioka et al. (1988).

In teleosts, GH has been implicated in somatic growth, development of seawater tolerance, regulation of thyroid hormone metabolism, gonadal steroid synthesis, and antifreeze

protein production (Wendelaar Bonga 1993). A single class of specific receptors for GH has been found in the liver, gills, intestine, and kidney of rainbow trout, the capacity of the liver being about 20-fold higher than that of the other organs (Sakamoto and Hirano 1991). The effects of GH seem to be partly mediated by insulin-like growth factors (Sakamoto et al. 1993; Wendelaar Bonga 1993), thyroid hormones (Leloup and Lebel 1993), and cortisol (Sakamoto et al. 1993). Plasma level of GH is affected by season (Barrett and McKeown 1989), photoperiod (Björnsson et al. 1995; McCormick et al. 1995), temperature (Barrett and McKeown 1989), feeding state (Farbridge and Leatherland 1992; Boujard et al. 1993; Reddy and Leatherland 1994), stress (Pickering et al. 1991), exercise (Barrett and McKeown 1989), and changes in salinity, and smoltification (see below). Important daily variations in plasma growth hormone concentration may occur, since Bates et al. (1989) recorded a 575% increase in plasma GH over a 2-h period in the middle of the night in juvenile coho salmon.

4.4.1.2. Possible involvement of growth hormone in smoltification

Plasma concentration of GH increases during smoltification, as shown in Atlantic salmon (Prunet et al. 1989; Bœuf and Le Bail 1990; Björnsson et al. 1995) and coho salmon (Young et al. 1989). In smoltifying Atlantic salmon, plasma GH concentration increased from 0.6 to 6.6 $ng \cdot mL^{-1}$ (Björnsson et al. 1995). Treatment of juvenile Atlantic salmon or amago salmon with ovine GH induces a number of changes associated with the parr–smolt transformation. Growth is enhanced, body silvering develops, seawater tolerance and gill Na^+K^+-ATPase activity increase, ultrastructural organization of gill chloride cell towards a seawater type appears, and preadaptation of the intestinal mucosa occurs (Miwa and Inui 1985; Prunet et al. 1994; Nonnotte et al. 1995). Positive, nonlinear correlations between hypoosmoregulatory ability and plasma GH concentration, and between growth rate and plasma GH concentration, were found in smoltifying Atlantic salmon (Björnsson et al. 1995). Growth rate increased rapidly with increasing plasma concentration of GH up to 2–3 $ng \cdot mL^{-1}$ at which point near maximal growth rate was reached (Björnsson et al. 1995). The effect of GH on the parr–smolt transformation is potent since a 2-week implantation of ovine growth hormone into juvenile nonmigratory brown trout induced a 4-fold increase in gill Na^+K^+-ATPase in freshwater and hypoosmoregulatory and growth capacities in seawater similar to those of fully smolted salmonids (Almendras et al. 1993). Due to a lower increase in Pco_2, a smaller difference between chloride and sodium influx and the absence of lactate elevation, as compared to untreated controls, GH treatment of rainbow trout and Atlantic salmon, moreover, reduced the acidosis associated with seawater transfer (Seddiki et al. 1995; Nonnotte and Bœuf 1995). Seddiki et al. (1995) proposed that GH reduced the decrease in diffusing capacity of gills induced by transfer to seawater and attenuated the decrease in oxygen affinity of haemoglobin caused by the metabolic acidosis.

The effect of GH on the gills may be more specific than that of thyroxine or cortisol. Whereas cortisol treatment stimulated proliferation and (or) differentiation of gill chloride cells in coho salmon pre-smolts, increasing both Na^+K^+-ATPase and Na^+K^+-independent ATPase activities, GH increased specifically Na^+K^+-ATPase activity without affecting chloride cell density (Richman and Zaugg 1987). This may be an advantage, since chloride cell proliferation or enlargement impede gas transfer through the gills (Bindon et al. 1994a, 1994b). Prunet et al. (1994) showed that GH implants in juvenile Atlantic salmon increased the number of seawater-type chloride cells and associated accessory cells, but decreased the number of freshwater-type chloride cells.

In Atlantic salmon, the two smoltification-associated GH peaks occurred at the same time as two peaks in plasma triiodothyronine, indicating a close relationship between GH and thyroid hormones (Prunet et al. 1989). In underyearling amago salmon, simultaneous treatment with thyroxine enhanced the effects of exogenous GH on growth, silvering, and seawater tolerance and was necessary for inducing a significant elevation of gill Na^+K^+-ATPase activity (Miwa and Inui 1985). GH stimulates hepatic conversion of thyroxine to triiodothyronine in rainbow trout (McLatchy and Eales 1990) and Leloup and Lebel (1993) demonstrated that triiodothyronine was necessary for the effect of GH on seawater adaptation of brown trout and rainbow trout.

Sakamoto et al. (1993) proposed that GH may act on the gills to promote their differentiation and induce their sensitivity to systemic IGF-I, possibly via local production of IGF-I. One possible mechanism could be the induction of IGF-I receptors synthesis by locally produced IGF-I. This effect of GH in inducing responsiveness to IGF-I may be a major effect of GH during smoltification, since gill filaments become sensitive to IGF-I *in vitro* as endogenous GH concentration increases and GH treatment early in smoltification induces sensitivity to IGF-I (Bern and Nishioka 1993). In accordance with this view, GH implants but not IGF-I implants increased seawater tolerance and gill Na^+K^+-ATPase activity in freshwater-adapted juvenile Atlantic salmon (McCormick 1996). After smoltification, sensitivity to IGF-I was lost even in growth hormone-treated fish (Bern and Nishioka 1993), indicating the complexity of interactions during this phase.

IGF-I implants or single injections of GH or IGF-I to freshwater-adapted juvenile Atlantic salmon failed to improve salinity tolerance, whereas the same treatments to fish acclimated to 12‰ seawater did (McCormick 1996). These results indicate that some differentiation of chloride cells, occurring as fish adapt to 12‰ seawater, may be sufficient for improving the potency of GH and IGF-I. Alternatively, high levels of prolactin in freshwater-adapted fish may have antagonized the effects of GH and IGF-I, since prolactin treatment abolished the seawater-adaptive effect of GH treatment in a dose-related manner in brown trout and rainbow trout (Madsen and Bern 1992). In contrast, cortisol improved the seawater-adaptive effect of GH and IGF-I (McCormick 1996). These hormones seem to act synergetically, GH increasing cortisol synthesis (Schreck et al. 1989) and gill cytosolic corticosteroid receptor concentration (Shrimpton et al. 1995), while corticosteroids stimulate endogenous GH release (Nishioka et al. 1988; Thakore and Dinan 1994).

4.4.1.3. Possible involvement of growth hormone in migration

The involvement of GH during migration of salmonids is suggested by the finding that wild migrating Atlantic salmon smolts had a mean plasma GH concentration 100 times that of nonmigrating wild parr or hatchery-reared smolts (McCormick and Björnsson 1994). Wild migrating coho salmon smolts caught at the river mouth similarly had higher GH concentration than parr caught in the river (Varnavsky 1992, cited in Sakamoto et al. 1993). Water flow challenge results in large and rapid increases in plasma growth hormone and fish placed in a floating net and towed downstream exhibit increases in plasma GH concentration (McCormick and Björnsson 1994). These observations are particularly interesting in relation to the stimulatory effects of GH on development of seawater tolerance (reviewed by Sakamoto et al. 1993), since they suggest that GH could mediate the positive influence of river migration on hypoosmoregulatory capacity of smolts, as shown in steelhead trout, chinook and coho salmon (Zaugg et al. 1985). The concomitant increase in plasma T_4 occurring in flow-challenged smolts (Youngson et al. 1986; Youngson and Webb 1992) could enhance the effect of increased plasma GH. In addition, increased plasma levels of GH

during migration could prepare the organism for energy mobilization during exercise bouts or environmentally imposed stress (Barrett and McKeown 1989).

Migration is usually associated with a decrease in condition factor, both in wild and hatchery-reared fish (Rodgers et al. 1987, 1984b; Ewing et al. 1994; Tipping et al. 1995). Growth hormone treatment increased body length more than body weight in a smoltifying species, the coho salmon, but not in brook trout, which is considered as nonsmoltifying (Skyrud et al. 1989; McCormick 1994). If there is a threshold condition factor under which migration may be induced, then a GH-induced decrease in condition factor accompanying smoltification could participate in regulating onset of migration.

4.4.1.4. Possible involvement of growth hormone during salinity changes

The transfer of salmonids to seawater is commonly associated with a transient increase in plasma GH concentration, due to increased pituitary synthesis and release (Sakamoto et al. 1993). The subsequent reduction of plasma GH to freshwater levels is in turn due to an increase in the metabolic clearance rate of GH, probably as the result of increased liver uptake (Sakamoto et al. 1990, 1993). In smoltifying salmonids, the change in plasma GH following seawater transfer seems to be strongly dependent on the developmental stage, since GH concentration doubled 24 h following seawater transfer of coho smolts in May, but showed no such change in March, April, June, or September (see Fig. 2 or Young et al. 1995). The period of "GH responsiveness" corresponded to a sharp decline in condition factor and no increase in plasma cortisol upon seawater transfer.

The transfer of rainbow trout from freshwater to 80% seawater was rapidly followed by an increase in GH receptor occupancy in the liver, while no changes in receptor occupancy was detected in whole gill or kidney tissues (Sakamoto and Hirano 1991). After 14 days, total binding sites in the liver were, moreover, increased. Sakamoto and Hirano (1991) suggested that seawater transfer rapidly stimulated internalization of GH/receptor complexes by liver cells, while upregulation of receptors was more time consuming. The changes in liver receptors following seawater transfer, as well as their high level in liver as compared to osmoregulatory organs, support a mediation by IGF-I of the osmoregulatory effects of GH following seawater transfer (Sakamoto and Hirano 1991). IGF-I is indeed expressed in the liver, gill, and kidney after GH treatment or seawater transfer and possesses osmoregulatory effects (McCormick et al. 1991; Sakamoto et al. 1993). The importance of GH in inducing sensitivity to IGF-I in osmoregulatory organs is discussed in section 4.4.1.2.

Freshwater transfer of seawater-adapted salmonids is in contrast accompanied by no significant changes in plasma GH concentration nor GH kinetics (Sakamoto et al. 1993).

4.4.2. Sex steroids

4.4.2.1. General aspects

Sex steroids are produced in gonads, interrenals (see McBride et al. 1986; Schreck et al. 1989), and brain (see Yamada et al. 1993) of salmonids. The three most frequently occurring estrogens in fish are estradiol-17β, estrone, and estriol (Woodhead 1975). The progestagene 17α-20β, dihydroxy-4-pregnen-3-one is present in the ovaries and is the most potent inducer of oocyte maturation in salmonids (Chieffi and Pierantoni 1987). The main androgen in females is testosterone; in males it is 11-ketotestosterone, which may be unique to teleosts (Lofts 1987).

Gonadotropins are the major regulators of sex steroid synthesis and seem to be under multihormonal control. GnRH as well as dopamine, vasotocin, norepinephrine, serotonin, and neuropeptide Y have been involved in the regulation of gonadotropin release (Redding and Patino 1993). Decreasing daylength is associated with increasing plasma concentration of gonadotropins (Hasler and Scholz 1983). Plasma levels of sex steroids increase dramatically during the spawning migration and decrease rapidly after spawning (Hasler and Scholz 1983; Lofts 1987). Water salinity and temperature may influence sex steroid production (Woodhead 1975; Lofts 1987). Circadian variations in plasma steroid levels have been evidenced in some teleosts (Chieffi and Pierantoni 1987).

The effects of sex steroids in fish are reviewed by Woodhead (1975) and Redding and Patino (1993). In short, absolute and relative amounts of different steroids probably play a major role in the differentiation of the gonads, the development of secondary sexual characters, gametogenesis, spawning, and many behavioral events linked to reproduction such as pheromone attraction and recognition of secondary sexual characters. They may also affect jumping behavior, motor activity, intestinal uptake of amino acids, growth rate, plasma lipid dynamics, temperature selection, and olfactory sensitivity. Sex steroids may exert either positive or negative feedback control on GnRH and gonadotropin secretion, depending on developmental stage (Woodhead 1975; Redding and Patino 1993).

4.4.2.2. Possible involvement of sex steroids in upstream migration

Experimental evidence of a role of gonadotropin or sex steroids in upstream migration of salmonids is presented by Hasler and Scholz (1983). Injection of chinook salmon gonadotropin into coho salmon at a nonmigratory stage increased locomotor activity of the fish and induced upstream movement (Hasler and Scholz 1983). Hasler and Scholz (1983) therefore suggested that the stimulation of upstream migration in salmon or trout by decreasing daylength is mediated by gonadotropins. The authors also suggested and provided some evidence that sex steroids or gonadotropins are responsible for increasing the olfactory sensitivity and behavioral response to imprinted odors of adult salmonids. They showed that sensitivity or behavioral response to home stream odor was low during the open-sea part of migration and after spawning, when plasma levels of sex steroids were low, and increased during the upstream migration concomitant with increasing plasma concentration of sex steroids. In both male and female Atlantic salmon, plasma levels of sex steroids indeed start to increase before the fish leave the ocean (Youngson and Mc Lay 1989). Hasler and Scholz (1983) proposed that gonadotropin or sex hormones act on the central nervous system to exert centrifugal control over the olfactory system, perhaps by desensitizing the receptors to all but the imprinted odor, or by altering central integration processes.

It is clear, however, that complete gonadal maturation is not necessary for upstream migration in a number of salmonids, such as Arctic char and brown trout, which migrate upstream even in years when they do not spawn. Nevertheless, the occurrence of gonadotropins or sex steroids, in concentrations insufficient to cause complete maturation but sufficient to participate in upstream migration, remains to be investigated. If sex steroids are involved in inducing upstream migration, they may be synthesized outside the gonads, since sterile male hybrids of brown trout × Arctic char may migrate upstream despite the absence of testicular tissue (Baggerman 1962). Since different species or individuals enter freshwater at varying stages of gonadal maturation (Woodhead 1975), major interactions with other factors must occur. Whereas king salmon entering the Sacramento River in spring

have gonads which have barely begun to develop, those entering the river in autumn have gonads which have achieved a much more advanced stage of maturation (Woodhead 1975).

4.4.2.3. Possible involvement of sex steroids in smoltification and downstream migration

Sex steroids may also play some role during smoltification and downstream migration of salmonids. Plasma estradiol-17β increased during smoltification of coho salmon, covarying with plasma concentration of thyroxine. Mean level reached a first peak early during smoltification, then a second one when 80% of the fish were morphologically classified as smolts (Sower et al. 1984). A similar pattern of variation of plasma estradiol and testosterone was observed by Yamada (1993) in smoltifying masu salmon. Such a pattern is similar to that described by Leatherland (1992) for thyroid hormones and suggests that sex steroids, like thyroid hormones, may influence both the process of smoltification and onset of migration. Plasma levels of testosterone in masu salmon were higher than those of estradiol (Yamada et al. 1993). However, testosterone seems to be mainly produced in the interrenals during smoltification and may be aromatized in the brain to estradiol. The local concentration of estradiol in the brain may therefore be far higher than reflected by its plasma concentration (Yamada et al. 1993). Some authors have failed to find any changes in plasma sex steroid concentration during smoltification (coho salmon: Patino and Schreck 1986; amago salmon: Nagahama et al. 1982). However, brain content of sex steroids was not estimated in these studies.

Yamada (1993) proposed that the two major effects of sex steroids during smoltification were (*a*) increased sensory perception, favoring imprinting and making the fish more sensitive to environmental cues regulating smoltification or onset of migration, and (*b*) stimulation of TSH and prolactin release from the pituitary, as well as increased peripheral deiodination of T_4 to T_3. Some effect on motor activity, growth, and lipid mobilization may exist as well. Sex steroids may also stimulate some gonadal development in coho smolts, although uptake of ^{3}H-estradiol-17β was much lower in gonads than in brain and pituitary at that time (see Sower et al. 1984). In chum salmon, a transient increase in estradiol and thyroxine occurred during emergence from the gravel (De Jesus and Hirano 1992). Since these fish migrate to the sea soon after emergence, these elevations may have been related to imprinting. Parhar et al. (1994) showed that during the seaward migratory period of chum salmon, there was a tendency of GnRH mRNA to increase in several ganglions of the CNS.

Whereas the effect of moderate levels of circulating sex steroids during smoltification remains uncertain, the administration of high doses of sex steroids clearly inhibits smoltification and downstream migration. Long-term administration of estradiol-17β, testosterone, 11-ketotestosterone or 11-ketoandrostenedione inhibited morphological, physiological, and behavioral features of smoltification in Atlantic, Baltic, or masu salmon. Silvering, black margin on the fins, decreased condition factor, number of chloride cells, gill Na^+K^+-ATPase, seawater tolerance, and downstream migration were inhibited by sex steroid treatment (Ikuta et al. 1987; Madsen and Korsgaard 1989; Berglund et al. 1994). Early sexual maturation may also interfere with smoltification and migration (Buck and Youngson 1982; Hoar 1988; Rydevik et al. 1989; Fängstam et al. 1993), although Lundqvist and Eriksson (1985) found little or no differences between immature Baltic salmon and precocious males in silvering, swimming behavior, and seawater tolerance during smoltification. Saunders et al. (1994) demonstrated that Atlantic salmon can develop as smolts following maturation as male parr the previous autumn provided they have reached the threshold size or energy level

for smolting before maturing as parr. In small individuals, the cost of maturation may be too high to allow another energy-consuming process, smoltification (Saunders et al. 1994). Sex steroids could interfere with major smolting hormones, such as the thyroid hormones (Madsen and Korsgaard 1989). Their effect in salmonids could thus be comparable with that in Japanese flounder, *Paralichtys olivaceus*, in which sex steroids inhibit the prometamorphic effect of thyroid hormones *in vitro* (De Jesus et al. 1993). An investigation of the relation between feeding state, smoltification, sexual maturation, thyroid hormones, and sex steroids would be interesting.

4.4.2.4. Possible involvement of sex steroids during salinity changes

According to Woodhead (1975), gonadal hormones do not seem to play a major role in the osmotic adaptations of salmonids during their migrations. Different salmonid species or individuals may enter freshwater at quite varying stages of gonadal maturation. Similarly, seawater entry may coincide with moderate gonadal development, as shown in coho salmon smolts (Sower et al. 1984), or with regressing gonads, as shown in spent adults or precocious parr (Buck and Youngson 1982; Woodhead 1975).

4.4.3. Melatonin

4.4.3.1. General aspects

Melatonin is produced by the pineal gland (epiphysis) and the retina of all vertebrates and is considered a major mediator of photoperiod in adjusting behavioral and physiological processes to daily and yearly rhythms (Wendelaar Bonga 1993). It is a tryptophan-derived indoleamide, which is synthesized and secreted mainly during the night (Zachmann et al. 1992). In fish, environmental light is directly sensed by the pineal through the skull and transduced into both a nervous and an endocrine signal (Meissl and Brandstätter 1992). The isolated pineal organ of rainbow trout responds within 5 min to sudden light exposure or darkness, by respectively decreasing or increasing melatonin synthesis (Gern et al. 1992). Daily cycles in plasma melatonin concentration or pineal melatonin content have been shown in rainbow trout, brook trout, Atlantic salmon, Baltic salmon, and Arctic char (Zachmann et al. 1992; Lindahl 1986; Randall et al. 1995; Bendiksen 1996). Although photoperiod is considered as the main regulator of melatonin secretion, temperature, seawater transfer, and local factors such as cAMP, catecholamines, adenosine, and possibly ANP also influence melatonin production (Zachmann et al. 1992; Falcón et al. 1992; Gern et al. 1984). An endogenous circadian rhythm of melatonin synthesis exists in northern pike, *Esox lucius*, and zebrafish, *Danio rerio*, but not in rainbow trout (Begay et al. 1998). Begay et al. (1998) suggested that this might be due to a genetic mutation in trout.

4.4.3.2. Possible involvement of melatonin in smoltification and migration

Smoltification and migration of salmonids are highly dependent on photoperiod. Manipulation of the photoperiod, which is known to alter melatonin secretion, may delay, accelerate, or synchronize within a population, aspects of smoltification (Wedemeyer et al. 1980; Hoar 1988). In particular, both hypoosmoregulatory capacity and migratory behavior are sensitive to changes in the photoperiod regime (Hoar 1988). Direction and rate of change of daylength seem to be more important photoperiodic cues than daylength *per se* (Wedemeyer et al. 1980). In Atlantic salmon, an abrupt increase in daylength advanced the smoltification-associated increase in plasma growth hormone, whereas exposure to continuous light or short days delayed and significantly reduced the increase in plasma growth

hormone (Björnsson et al. 1995; McCormick et al. 1995). Atlantic salmon exposed to reciprocal photoperiod, decreasing daylength from early March and increasing daylength from late June, developed a high condition factor during the normal period of smoltification, showed poor growth and low efficiency of food conversion after transfer to seawater, and were less sensitive to external stimuli than those under natural or constant photoperiod (Saunders and Henderson 1970, cited in Hoar 1988). In contrast, the pituitary–thyroid axis may be relatively insensitive to photoperiod, since body silvering, thyroid histology, and plasma levels of thyroid hormones were not significantly altered by changing photoperiod (Hoar 1988; McCormick et al. 1995).

Under natural conditions, seaward migration occurs most often during conditions of low light intensity (Hasler and Scholz 1983; Northcote 1984), when melatonin release can be expected to be high. The involvement of the pineal gland in some behavioral responses to light is strongly indicated by the observation that sockeye smolts were able to maintain their normal negative phototactic response after blinding but became indifferent to light when the pineal was destroyed (Hoar 1955, cited in Smith 1985).

In addition to photoperiod, smoltification and onset of migration also depend on a number of external and internal factors (cf. Chapter 1 and review by Hoar (1988)). The pineal gland may be part of a network integrating all these signals. Melatonin may act on migration and smoltification both directly and indirectly, by modulating nervous and hormonal signals. Melatonin binding sites have been found in several brain areas of Atlantic salmon, high levels being primarily associated with the hypothalamus and the optic tectum with associated visual centers (Ekström and Vanecek 1992). These localizations are consistent with a role for melatonin in modulation of the activity of a variety of neuroactive peptides, such as somatostatin, corticotropin-releasing factor, growth hormone-releasing factor, vasotocin, isotocin, and a role in modulation of the sensory processing that occurs in teleostean optic tectum (Ekström and Vanecek 1992). In rainbow trout, melatonin treatment may inhibit swimming activity, induce blanching, and modulate neural activity of the pineal gland (see review by Zachmann 1992).

4.4.3.3. Studies on the Arctic char

Anadromous Arctic char in northern Norway and Svalbard are exposed to unusual photoperiodic conditions. Arctic char from the Hals River system (70°N) remain under ice cover from December until early June (Strand and Heggberget 1994), while at Spitsbergen Island (74–80°N), Arctic char may remain under ice cover from the end of September until the beginning of August (Nilssen et al. 1997). At ice breakdown, the fish are exposed to continuous daylight, although the intensity of the light and its spectral composition may show daily variations (Stokkan et al. 1994; Bendiksen 1996). No publications were found concerning melatonin secretion in fish under such conditions. The reindeer, *Rangifer tarandus tarandus*, and the emperor penguin, *Aptenodytes forsteri*, which also live in Arctic or Antarctic regions, show little or no daily variations in plasma melatonin secretion both in midsummer and midwinter (Miché et al. 1991; Stokkan et al. 1994). It could therefore be expected that this remains true for the Arctic char. In reindeer, however, as soon as daylength began to decrease, the daily secretion of melatonin increased from an annual minimum in June–July to an annual maximum in August (Stokkan et al. 1994). Such sudden increase in melatonin secretion, occurring some time after the summer solstice, could represent a potent signal for upstream migration of anadromous Arctic char.

Development of seawater tolerance in the Arctic char is regulated by photoperiodic cues (Arnesen et al. 1992). It is known from other salmonid species that a period of

darkness prior to increasing daylength may not only synchronize smoltification within a population but also accelerate the changes that occur in the subsequent period of lengthening photoperiod (Hoar 1988). In the north of Norway, the long winter conditions with ice cover, followed by the rapid increase in daylength and ice breakdown, may represent an extreme case of such an effect. Anadromy in Arctic char is limited to most northern areas and southern Arctic char may show anadromous behavior when released in these northern areas (Nordeng 1983). It has been speculated earlier in this review that temperature or food availability could explain this behavior. The possibility that anadromy in Arctic char is dependent on a sharp contrast between summer and winter light conditions should be investigated as well.

The pineal gland of Arctic char may respond to changes in the intensity of light occurring during summer days, since *in vitro* studies with rainbow trout indicate that the pineal gland is capable of giving a graded response to illumination and responds to relative changes in illumination rather than to absolute values (Gern et al. 1992). Interestingly, whereas light inhibited melatonin synthesis in the rainbow trout and Atlantic salmon during the whole period of illumination (Gern et al. 1992; Randall et al. 1995), melatonin synthesis in Arctic char progressively escaped from the inhibiting effect of light. Under experimental *in vivo* conditions, a sharp decrease in plasma melatonin concentration following light exposure was followed by a slow increase in melatonin concentration towards night levels during continued light exposure (Bendiksen 1996). This indicates a strong ability of the Arctic char pineal gland to adapt to illumination, similar to that of the retina, thus increasing its ability to respond to contrasting light conditions, either at high or low light intensities. Evidence of light adaptation of the pineal gland are dealt with in Meissl and Brandstätter (1992).

The pineal gland of Arctic char may also respond to changes in spectral activity of light during summer days of continuous illumination. In particular, Arctic char seem to be more sensitive than rainbow trout to red light, for melatonin production (Bendiksen 1996; Meissl and Brandstätter 1992; Gern et al. 1992). In brown trout, red light is able to entrain the circadian activity rhythm (Borja 1990). These observations suggest that the effect of dim red light should be tested on each species or stock before it is used for providing necessary light for experimental observation or sampling during the scotoperiod in fish.

In constant darkness, melatonin synthesis *in vivo* in Arctic char remained high for at least 24 h (Bendiksen 1996). Similar results have been obtained *in vitro* in rainbow trout (Zachmann et al. 1992). Begay et al. (1998) showed that two enzymes responsible for melatonin synthesis lacked a circadian rhythm in rainbow trout and suggested it might be due to a single genetic mutation. In contrast, several nonsalmonid species such as pike, goldfish, and white sucker, *Catostomus commersoni*, are able to maintain a circadian melatonin secretion when kept in constant darkness and temperature conditions (Zachmann et al. 1992). Thus salmonids may depend more tightly upon external factors such as light and possibly temperature for melatonin secretion, which may be a major aspect in their ability to rapidly adapt to changing environmental conditions.

4.4.4. ANP-like peptides

4.4.4.1. General aspects

A-type Natriuretic Peptide (ANP), also called atrial natriuretic peptide, atrial natriuretic factor (ANF) or atriopeptine, was the first member of a family of natriuretic peptides to be identified in the early 1980s. B-type (originally identified in the brain), C-type, and recently V-type (identified only in the ventricle of eel) natriuretic peptides (BNP, CNP, and VNP,

respectively) were later isolated and sequenced in various vertebrate species, including fish (Takei 1993). Among vertebrates, the 22–32 amino acid long sequences seem to be best conserved for CNP and least conserved for BNP (Takei 1993). These peptides are synthesized in a number of tissues, including the brain (where they act as neuromediators), the heart (from which they are released into blood), and the thymus (where they may act as immunomodulators) (Vollmar and Schulz 1990; Wendelaar Bonga 1993). ANP synthesis in mammals is stimulated by glucocorticoids and thyroid hormones, while ANP release from the atrium is stimulated by atrial stretch, increased atrial tension, central nervous stimulation, and possibly increased plasma sodium concentration (Evans 1990; Christensen et al. 1988; Franci et al. 1992).

Immunoassays based on human ANP have been largely used for the determination of ANP-like immunoreactivity in a variety of fish species (Olson 1992). Such assays may underestimate the actual natriuretic peptide content of teleost tissues and body fluids (Wendelaar Bonga 1993) and it is at present uncertain which natriuretic peptides are measured (Takei 1993). According to Westenfelder et al. (1989), rainbow trout ANP closely resembles human ANP, suggesting that radioimmunoassay based on human ANP may be adequate for measuring salmonid ANP. However, natruretic peptides have not yet been sequenced in any salmonid species. Since tissue receptors are known to be very sensitive to minute structural changes of the peptides (Olson 1992), antibodies may be as well. In the eel, the radioimmunoassay for human ANP has been shown to measure eel VNP rather than eel ANP (Takei 1993). In sharks, only one of four commercial radioimmunoassay kits indicated a change in plasma "ANP" in volume expanded sharks (Olson 1992).

In mammals, under experimental conditions, ANP is a potent vasodilator and produces natriuresis and diuresis, lowering blood pressure (Evans 1990). It acts both directly on vessels and kidneys and indirectly by inhibiting the release of ADH and the renin-angiotensin-aldosterone system (Evans 1990). Its importance for volume homeostasis during pathological states, such as heart failure, is well recognized, while its physiological roles in normal subjects are more uncertain (Lohmeier et al. 1995; Volpe 1992).

4.4.4.2. Possible involvement of ANP during salinity changes

The few studies concerned with ANP in salmonids suggest that natriuretic peptides may play a role in the adaptation of migrating salmonids to different salinities by acting on osmoregulatory and cardiovascular structures. Plasma concentration of ANP, as measured by radioimmunoassay for human ANP, increased in Atlantic salmon and rainbow trout following seawater transfer (Smith et al. 1991). High levels were maintained for at least 3 weeks in the rainbow trout. Parr of Atlantic salmon showed a greater increase in plasma ANP than smolts, which may reflect the greater circulatory stress, the increased dorsal aortic blood pressure and the hypoxia experienced by the parr. In the cited study, however, sodium, chloride, and magnesium concentrations following transfer were similar in parr and smolts. Rainbow trout fed a high-salt diet, 12% NaCl, also showed increased plasma concentration of ANP, suggesting that ANP may be involved in both salt and volume regulation in salmonids (Smith et al. 1991).

ANP administration may have either a pressor–depressor effect, a depressor effect, or no effect on blood pressure in trout, probably depending on the rate of ANP administration (Olson 1992). In Atlantic salmon, eel ANP had a relaxing effect on a major branch of the dorsal aorta but no effect on the ventral aorta, when both vessels were precontracted with acetylcholine (Sverdrup and Helle 1994). Continuous infusion of rainbow trout with rat ANP lowered primary blood volume by only 23% and extracellular fluid volume by 36%

(Olson 1992), suggesting that fluid was transferred from intercellular spaces or secondary vessels into primary vessels. Thus, ANP may regulate blood flow by exerting specific effects on major blood vessels on both sides of the gills (Sverdrup and Helle 1994) and possibly exchanges between primary and secondary circulatory systems (for review on the secondary circulatory system, see Steffensen and Lomholt 1992). Such regulation may be of importance during adaptation to different salinities.

Injection of mammalian ANP or homologous heart extracts to rainbow trout increases urine flow rate, urinary sodium, potassium, and chloride concentrations (Duff and Olson 1986). This effect occurs even in fish showing a hypotensive response to ANP, or in perfused trout kidney in which perfusion pressure is held constant (Olson 1992; Takei 1993). Thus a hypertensive-associated increase in glomerular filtration rate is not necessary for the renal effect of ANP in salmonids.

In addition to promoting renal excretion of sodium and chloride, ANP may induce chloride extrusion through the gills. Mammalian ANP stimulated chloride extrusion from the opercular epithelium of seawater-adapted killifish and from the rectal gland of the spiny dogfish, *Squalus acanthias* (Takei 1993). In gill cells isolated from seawater-adapted rainbow trout, mammalian ANP stimulated production of intracellular cGMP (Olson 1992), which seems to mediate most of the effects of ANP, both in mammals and fish (Evans 1990). This effect was absent in gills from freshwater-adapted rainbow trout, indicating that ANP activity may require seawater-specific structures or mediators. Similarly, ANP stimulated *in vitro* and *in vivo* cortisol secretion by the rainbow trout in seawater, but not in freshwater (Arnold-Reed and Balment 1991). ANP-like materials have been identified in the chromaffine cells of all classes of vertebrates including fish (Wolfensberger et al. 1995), which indicates that ANP may act as a paracrine signal for steroid synthesis in the interrenal of salmonids.

ANP may counteract drinking behavior by inhibiting the renin-angiotensin system (Smith et al. 1991). In Atlantic salmon parr transferred to seawater, plasma ANP was high, whereas renin activity and drinking activity was low. In contrast, smolts transferred to seawater displayed lower plasma ANP than the parr, but higher plasma renin activity and drinking rates (Smith et al. 1991). Mammalian ANP has been shown to inhibit salt absorption from the intestine of seawater-adapted flounder (O'Grady 1989; O'Grady et al. 1985), but such effect has not been studied in salmonids.

In summary, ANP may be involved in hypoosmoregulation and possibly cardiovascular adjustments in migrating salmonids. The stimulus for ANP release in seawater may be increased blood volume and (or) increased blood osmolarity (Olson 1992). However, the structure of salmonid natriuretic peptides remains to be identified to validate the present conclusion. Recently, Takei (1993) showed, by using homologous radioimmunoassays specific to each peptide, that plasma concentrations of ANP, CNP, and VNP were in fact higher in freshwater eel than in seawater eel, suggesting a role in freshwater adaptation rather than seawater adaptation of eel. As stated by Takei (1993), we are still "far from understanding the roles of ANP, VNP, and CNP in fishes."

4.4.5. Insulin

4.4.5.1. General aspects

As in mammals, insulin seems to be a major anabolic hormone in fish, stimulating storage of proteins, lipids, and carbohydrates (reviewed by Mommsen and Plisetskaya 1991). In most carnivorous and omnivorous fish, including salmon, amino acids seem to be more

potent stimulators of insulin secretion than glucose. Insulin may channel nutrients towards lipid and protein stores rather than glycogen stores. In fasted rainbow trout, oral ingestion of a high-protein diet or insulin injection depressed gluconeogenesis. Insulin consistently increased amino acid uptake and protein synthesis in all species tested. Insulin inhibited triacylglycerol lipase and tended to decrease plasma content of free fatty acids in coho salmon. In rainbow trout, glucose carbons were channeled into muscle lipid (Mommsen and Plisetskaya 1991).

Plasma levels of insulin in fish tend to be higher than in mammals (Mommsen and Plisetskaya 1991), are positively correlated with size or growth rate (Sundby et al. 1991), and decrease during fasting (Mommsen and Plisetskaya 1991).

4.4.5.2. Possible involvement of insulin in migration

During the spawning migration of pink salmon and Atlantic salmon, insulin levels progressively declined from high levels in seawater to minimal levels during spawning (Murza et al. 1991; Mommsen and Plisetskaya 1991). The rate of decrease was different in males and females (see Mommsen and Plisetskaya 1991), which may be related to different requirements for gonadal growth. Decreasing insulin levels could result from cessation of feeding, and allow for increasing gluconeogenesis, plasma glucose, and liver glycogen stores.

4.4.5.3. Possible involvement of insulin in smoltification

Plasma insulin concentration increased during early smoltification of coho salmon (Mommsen and Plisetskaya 1991) and Atlantic salmon (Mayer et al. 1994), peaking about two months before smoltification was achieved and prior to the surge of thyroxine, growth hormone, prolactin, and cortisol (Dickhoff 1993). Plasma insulin concentration then declined during the middle and late stages of smoltification, as a result of decreased secretion rate rather than a decrease in hormone synthesis, thus leading to an accumulation of insulin in secretory granules in the pancreas (Mommsen and Plisetskaya 1991). In both species, insulin levels rose again around the time the fish were ready to be released into seawater (Mommsen and Plisetskaya 1991). The elevation of plasma insulin during early smoltification may function to fortify metabolic stores of lipid, protein, and carbohydrate (Dickhoff 1993). In wild fish with a low condition factor at onset of smoltification, this period of anabolism may be visualized as a transient increase in condition factor before the typical smoltification-associated decrease in condition factor occurs, as shown in wild coho salmon (Shrimpton et al. 1994). During the middle and late stages of smoltification, lipolysis and glycogenolysis are activated in order to fuel the parr–smolt transformation and reduced plasma insulin may be crucial to allow this transition from an anabolic to a catabolic state (Mommsen and Plisetskaya 1991). As smoltification is achieved, release of insulin increases, turning metabolism back to an anabolic state. The precise timing of this increase in relation to migration and seawater entry is unknown. It is clear that it responds to seasonal or endogenous cues since it occurs even when smolts are kept in freshwater and fed to satiation (Mayer et al. 1994). Under natural conditions, smolts migrate from nutrient-poor areas to nutrient-rich areas and food intake associated with seawater entry may function as an effective triggering mechanism for massive release of stored insulin. Under hatchery conditions, such a signal coordinating insulin release and seawater transfer may be absent. Proper timing of seawater entry of smolts is known to influence not only survival but also growth potential of salmonids, inappropriate timing being sometimes associated with growth inhibition and arrested development, known as "stunting" (Folmar et al. 1982).

It is tempting to suggest that "appropriate timing" includes a close temporal association between massive insulin release and seawater transfer, since growth hormone increases following seawater transfer of smolts (Young et al. 1995) and effective stimulation of growth may "require the concerted action of at least three components: insulin, growth factors, and growth hormone" (Mommsen and Plisetskaya 1991).

4.4.5.4. Possible involvement of insulin during salinity changes

Insulin affects the ionic composition of fish red blood cells, but present knowledge does not point to a specific role for insulin in development of hypoosmoregulatory capacity nor to any effect on ion flux rates in gills (Mommsen and Plisetskaya 1991; Mayer et al. 1994).

4.4.6. Others

A number of other hormones have been involved in processes that may be of importance during migration of anadromous salmonids. Although most studies deal with nonsalmonid species, such knowledge may provide an important background to test their involvement in salmonid migration. For example, increased secretion of urotensin I and II following seawater transfer may participate in increasing intestinal salt and water uptake, blood pressure, and plasma cortisol concentration (Wendelaar Bonga 1993). Rapidly acting hormones and neuromediators may modulate ion exchanges immediately following salinity changes. For example, chloride extrusion through skin and opercular epithelium of seawater fish is rapidly increased by epinephrine (β-effect), glucagon, urotensin I, vasointestinal peptide, leukotrienes, and rapidly decreased by epinephrine (α_2-effect), urotensin II, acetylcholine, and prostaglandin E_2 (see Marshall 1995). Increased plasma concentration of catecholamines in late smoltification or during hypoxia and acidosis may affect blood circulation, respiration, and energy balance (Hoar 1988; Wendelaar Bonga 1993). Angiotensin II stimulates drinking in most fish examined so far and may be antidiuretic in rainbow trout (Takei 1993). The reader is referred to Olson (1992), Takei (1993), and Wendelaar Bonga (1993) for further details. For studies on calcitonin and calcium regulation, see Watts et al. (1975), Flik and Perry (1989), and Björnsson et al. (1989).

Conclusions

The main difficulty we are facing when studying salmonid migration may be that salmonids represent a group of vertebrates with a very high degree of plasticity. They may migrate to the sea from soon after emergence from the gravel to more than 10 years later. The migration may be rapidly directed to the sea or may include several steps, sometimes lasting several months or years. Onset of migration may occur in response to a large diversity of abiotic and biological cues. Preadaptation to seawater may be minimal or complete. They may return to freshwater as immature or mature fish, after a few weeks to many years. Accordingly, physiological changes associated with the diadromous migration of salmonids are very variable. Therefore, extreme care must be taken not to generalize results obtained in one study to other species, stocks, or even to the same stock under slightly different conditions.

In such a situation, one may feel that experimental results lose much of their interest. In a way, they do, but in another, they gain more importance. The plasticity of salmonids makes research on this group particularly important in a general biological perspective. Comparative studies on salmonids may allow one to determine which elements are conserved, therefore probably major, essential steps, and which elements are exchangeable, therefore perhaps not as basic. Comparing these with other organisms may allow the discovery of fundamental mechanisms involved in plasticity.

The putative effect of thyroid hormones in triggering downstream migration may approach such a central place, at least in salmonids. An increase in plasma thyroxine may occur in relation to the new moon (Grau et al. 1981), increased water flow (Youngson et al. 1983), and abrupt changes in water temperature or in water composition (Lindahl et al. 1983; Nishioka et al. 1985). Each of these factors has been associated with onset of migration (cf. section 1.1.1) and increased plasma thyroxine could be a common signaling pathway for these different triggering factors. Thus a peak in thyroid hormones could be one major factor in inducing downstream migration. Once this is suggested, negative results deserve particular attention and criticism. For example, sampling intervals that are too long, poor synchrony between individuals, and inconsistency in the diurnal timing of sampling, are examples of situations were a short peak in thyroid hormones may exist without being registered. This illustrates the importance of rigorous performance and thorough reporting of methodological aspects.

Modesty in interpreting hormone data is also required due to the complexity of hormone action. The distribution and metabolism of thyroxine, for example, change during smoltification and according to feeding state. Moreover, thyroid hormones interact with other hormones, which are not secreted at a constant rate during smoltification. Thus, similar changes in plasma hormone concentration will obviously have different effects at different time periods. Transfer between freshwater and seawater is associated with changes in water flux between different compartments, changes in osmolarity and ionic composition, and often temperature. These factors may affect the binding affinity of hormones to plasma proteins and membrane receptors, and their plasma concentration. The extent and physiological consequences of such changes remain largely unexplored.

Biorhythms are also a major aspect of salmon migration, which is a typical seasonlly related event. In addition to the methodological importance of assessing seasonal and daily changes in hormone concentrations, chronobiology has important physiological and practical correlates. The effects of seasonal changes in photoperiod and temperature on smoltification have been documented. The effects of seasonal changes in feeding have

apparently not been studied, although feeding is likely to be a potent zeitgeber for at least some smoltification-associated changes. Such an effect is worth studying since the addition of food to photoperiod and temperature as zeitgebers may increase the synchronization of the different elements of smoltification, which is not always satisfactory under hatchery conditions. Studies on circadian variations are also interesting since they support the possibility that the regulation of salmonid migration may include temporal relations between the circadian variation of several hormones, as described by Meier (1980) in apparently simpler models, the killifish and the sparrow. Daily variations in plasma concentration of several hormones have been demonstrated in salmonids, including melatonin (Randall et al. 1995), cortisol (Audet and Claireaux 1992; Boujard et al. 1993), thyroxine (Audet and Claireaux 1992; Boujard et al. 1993), growth hormone (Bates et al. 1989; Boujard et al. 1993), and arginine vasotocin (Kulczykowska and Stolarski 1996). The daily cycles of thyroxine and cortisol have been shown to vary from month to month (Audet and Claireaux 1992). The migratory model of Meier (1980) included prolactin and corticosterone. Studies of the plasma concentration of prolactin in salmonids have so far failed to identify any daily rhythm (Prunet and Bœuf 1989). The structurally related growth hormone could be an interesting alternative candidate, since its plasma concentration may show a particularly marked daily rhythm. In juvenile coho salmon, a sixfold increase in plasma growth hormone over a 2-h period in the middle of the night was recorded (Bates et al. 1989). Moreover, experiments on killifish were based on ovine prolactin, which now is known to bind similarly to prolactin receptors and growth hormone receptors in tilapia (Prunet and Auperin 1994); thus ovine prolactin may have induced growth hormone effects in the killifish.

As a final example of the importance of integrative approaches, the effect of migration on development of seawater tolerance can be recalled. Hatchery-reared smolts released in a river may increase seawater tolerance during their downstream migration to the same level as smolts transferred to seawater (Zaugg et al. 1985). Thyroxine, cortisol, and growth hormone, which all are involved in development of seawater tolerance and are generally higher in migrating individuals, may be central in this process. The determination of which factors in migration cause this increase in seawater tolerance may have important correlations in hatchery practice and lead to a better understanding of wild populations. Ecological, behavioral, physiological, and cellular mechanism must be considered together.

All new studies are important to the complex and fascinating field of the physiology of diadromy in salmonids. They might not be more than a drop in the ocean, but at least a drop in a splendid ocean.

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